Evidence Against a Role of Acetaldehyde in Electroencephalographic Signs of Ethanol-Induced Intoxication

Abstract. *Acetaldehyde, the proximate metabolite of ethanol, when injected intravenously in rats produced electroencephalogram (EEG) changes similar to those observed after ethanol administration; that is, low doses activated the cortical EEG and higher doses caused activation followed by synchronization. However, when acetaldehyde was administered as a continuous infusion to simulate production of ethanol-derived acetaldehyde, only synchronization occurred, and then only at the higher doses. At low infusion dosage when the EEG was unaffected, concentrations of acetaldehyde in the blood were equal to or greater than those which occur during intoxication. Thus, acetaldehyde by itself cannot account for ethanol-induced EEG synchronization.*

Some neurophysiological and behavioral consequences of ethanol administration may not result from the actions of ethanol itself, but from those of its metabolites. Experimental data have been interpreted to indicate that ethanol-derived acetaldehyde mediates some of the pharmacological and biochemical effects seen during short- or long-term ethanol administration (*1*). Single injections (intraperitoneally or intravenously) of large doses of acetaldehyde cause behavioral effects similar to those after ethanol administration, such as ataxia and hypnosis in rats (*2*) and depression of locomotor activity in mice (*3*).

A knowledge of acetaldehyde effects on the electroencephalogram (EEG) could provide a broader data base on which to make comparisons between the effects of ethanol and acetaldehyde. The effect of ethanol administration on the spontaneous EEG has been well documented both in experimental animals and in humans. Ethanol generally deactivates the EEG (that is, causes high voltage, slow activity), but it can cause brief periods of activation (low voltage, fast activity) soon after injection (*4*).

There are very few data on the EEG effects of acetaldehyde, and most of these data were derived from experiments in which animals were injected with large doses of the compound that create much higher concentrations of acetaldehyde in the blood than develop after intoxicating doses of ethanol. Single injections of low doses (1.5 to 3.0 mg/kg, intravenously) of acetaldehyde in rabbits failed to alter the EEG (*5*), but higher doses (20 to 40 mg/kg) in cats produced EEG changes that superficially resembled the biphasic response commonly seen after ethanol administration (*6*). However, we thought that this information should be interpreted reservedly, since a slightly higher dosage (50 mg/kg) produced severe and sometimes fatal convulsions in rabbits (*7*). The EEG responses could result from a direct toxicity of very high concentrations of acetaldehyde or from an indirect sympathomimetic action (*8*).

We investigated the effects of acetaldehyde on the EEG by testing three hypotheses: (i) that a single injection of acetaldehyde produces the same dose-related biphasic EEG effect that is caused by ethanol; (ii) that different effects are produced with continuous infusion of acetaldehyde (because of the short half-life of acetaldehyde, a sustained concentration cannot occur after a single injection) (*1*, *9*); and (iii) that some acetaldehyde effects on the EEG are mediated by peripheral influences, either neural or hormonal.

The surgery, consisting of tracheal intubation, implantation of epidural recording electrodes (*10*), and jugular catheterization, was carried out on 29 male Wistar rats anesthetized with ether. Eight of these rats were cervically transected after completion of the surgical procedures. Five animals were adrenalectomized 12 hours prior to the experiments. At the completion of surgery, all wound margins were locally anesthetized (*11*), ether was removed, a paralyzing agent was administered (*11*), and artificial respiration was started. Experimentation began after a lapse of at least 50 minutes.

Spontaneous EEG activity was recorded from each animal by means of a conventional eight-channel EEG recorder (*12*). One channel of EEG was derived from two bilateral frontal electrodes, a second from the two bilateral parietal electrodes. Each of these channels was led to three different amplifiers so that each signal was monitored simultaneously at three different passbands (*13*). All EEG data were analyzed visually and independently by J.A.M. and W.R.K. (*14*). Heart rate was monitored in all experiments (*15*). A control EEG was recorded before acetaldehyde was injected or infused (*16*). Fifteen minutes were always allowed to elapse between successive injections or infusions.

In separate experiments, two rats were used for measuring concentrations of acetaldehyde in the blood before, during, and after infusions of the highest and lowest acetaldehyde doses (12.5 and 3.1 mg/kg per minute for 4 minutes, respectively). Blood samples were taken from a carotid catheter, and acetaldehyde concentrations were determined by a gas chromatographic technique adapted from the work of others (*17*).

In the single-injection experiments, physiological saline produced no apparent responses in seven of the rats tested. Of the three rats that did respond, two had a mild, brief EEG activation and one had such activation followed by a short period of deactivation. Acetaldehyde, at the lower doses, caused a short-latency,

Table 1. Changes in the EEG after administration of acetaldehyde in rats subjected to cervical transection and adrenalectomy. Latencies and durations were measured in seconds.

Rat	Single injection (25 mg kg^{-1})						Continuous infusion (12.5 mg kg^{-1} min^{-1})			
	Activation		Amplitude flattening		Overt synchrony		Decreased activation		Overt synchrony	
	Latency	Duration	Latency	Duration	Latency	Duration	Latency	Duration	Latency	Duration
					Transected rats					
1									120	829
2			13	32	45	231	34	40	74	426
3			15	22	182	200			350	320
4	4	15			35	52	83	12	95	347
5	5	15					175	61	236	54
6	5	17			50	172	50	75	125	65
7									61	411
8									110	351
					Adrenalectomized rats					
1	5	8			25	195	40	90	130	370
2					21	218	55	66	121	310
3									138	179
4							122	43	165	234
5					17	647			178	149

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short-duration activation in all rats. Larger doses generally caused more complex responses, with most animals having an initial activation that was often followed by an episode of near-isoelectric amplitude depression succeeded in turn by EEG synchronization. When amplitude depression did not occur after an injection, synchronization developed immediately after the activation (Fig. 1).

At doses higher than 6.25 mg/kg, the latency and duration of amplitude depression did not appear to vary appreciably with dose; however, there did appear to be a relation between dose and the number of animals with amplitude depression (Fig. 2). Synchronization effects were dose-related in terms of incidence, but not latency or duration.

The results partially confirm the results obtained by Ogata (*6*) in that low doses of acetaldehyde activated the EEG. Several aspects of the data support the hypothesis that activation occurs with low concentrations of acetaldehyde in the brain. Activation alone occurred at the lowest acetaldehyde dosage used, and occurred with approximately the same latency and duration at all doses employed. However, the absence of EEG activation in the continuous infusion study strongly militates against the possibility of acetaldehyde having a role in the production of ethanol-induced activation. During infusion, blood and brain concentrations of acetaldehyde more closely approximate the development of ethanol-derived acetaldehyde concentrations, because they increase slowly (concentrations in our rats still seemed to increase at the end of infusion).

The EEG flattening after acetaldehyde injection undoubtedly reflects acetaldehyde toxicity. Concentrations of acetaldehyde in the brain probably reached a maximum during the time of amplitude depression. The EEG synchronization observed after acetaldehyde injection does not, by itself, permit inferences about possible pharmacologic actions of ethanol-derived acetaldehyde. In approximately half of the rats with synchronization the response was preceded by amplitude depression; therefore, we do not know if synchronization was a direct result of acetaldehyde or if it reflected recovery from the prior amplitude depression.

Injections of acetaldehyde slowed heart rate, beginning at injection and returning toward the normal baseline level within 20 seconds. The bradycardia was progressively severe with increasing dosage of acetaldehyde, with a maximum decrease of approximately 33 percent after a dose of 50 mg/kg. Electrocardiogram abnormalities (extrasystoles, dropped beats, and episodes of pronounced arrhythmia) were also observed in association with the bradycardia, but the incidence of paroxysms did not appear to be correlated with dose.

The role of peripheral influences in mediation of the EEG responses was studied in other rats that were either cervically transected or adrenalectomized. Usng a dose that caused the full spectrum of changes in intact animals without causing signs of excessive toxicity, we found that transection did not appear to alter the pattern of EEG responses to single injections (Table 1); however, adrenalectomy seemed to diminish the activation and amplitude-depression, suggesting that those phenomena may be related to interaction with catecholamines in the brain and adrenal gland. Control injections of saline in each animal of both groups had no effect. Acetaldehyde injection (25 mg/kg) in cervically transected and adrenalectomized rats also produced decreases in heart rate with maximum changes of 48 and 37 percent, respectively.

Somewhat different EEG responses occurred during continuous infusion of acetaldehyde in intact rats (Fig. 1). All rats infused at the two higher doses showed a clear deactivation that was not evident with the control dose or with the

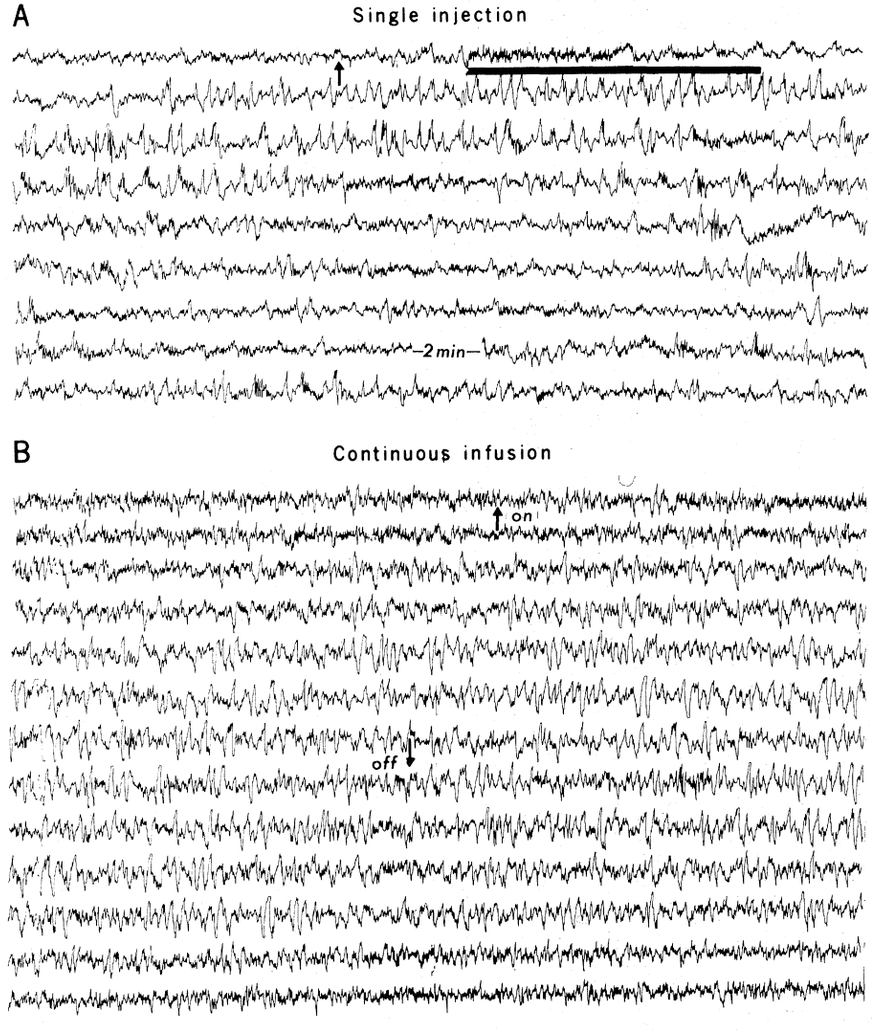

Fig. 1. Comparison of the EEG (0.3 to 90 Hz band-pass) responses to (A) a single injection and (B) continuous infusion of acetaldehyde. Tracings from one pair of electrodes are shown in a raster display reading left to right. Arrows indicate point of injection and beginning or end of infusion. (A) Single injections of high doses (above 6.3 mg kg^{-1}) produced a brief EEG activation (underlined portion of record), followed by synchronization, sometimes with an intervening period of EEG flattening (not shown); a return toward the normal, preinjection activity is evident in the latter part of the last trace. (B) Continuous infusion at 6.3 or 12.5 mg kg^{-1} min^{-1} caused marked synchronization that developed after about 1 minute and persisted about 3 minutes after infusion was terminated (lower doses were without effect); return toward normal, preinjection activity is evident in the last two traces. Calibration marks: 100 μV and 1 second.

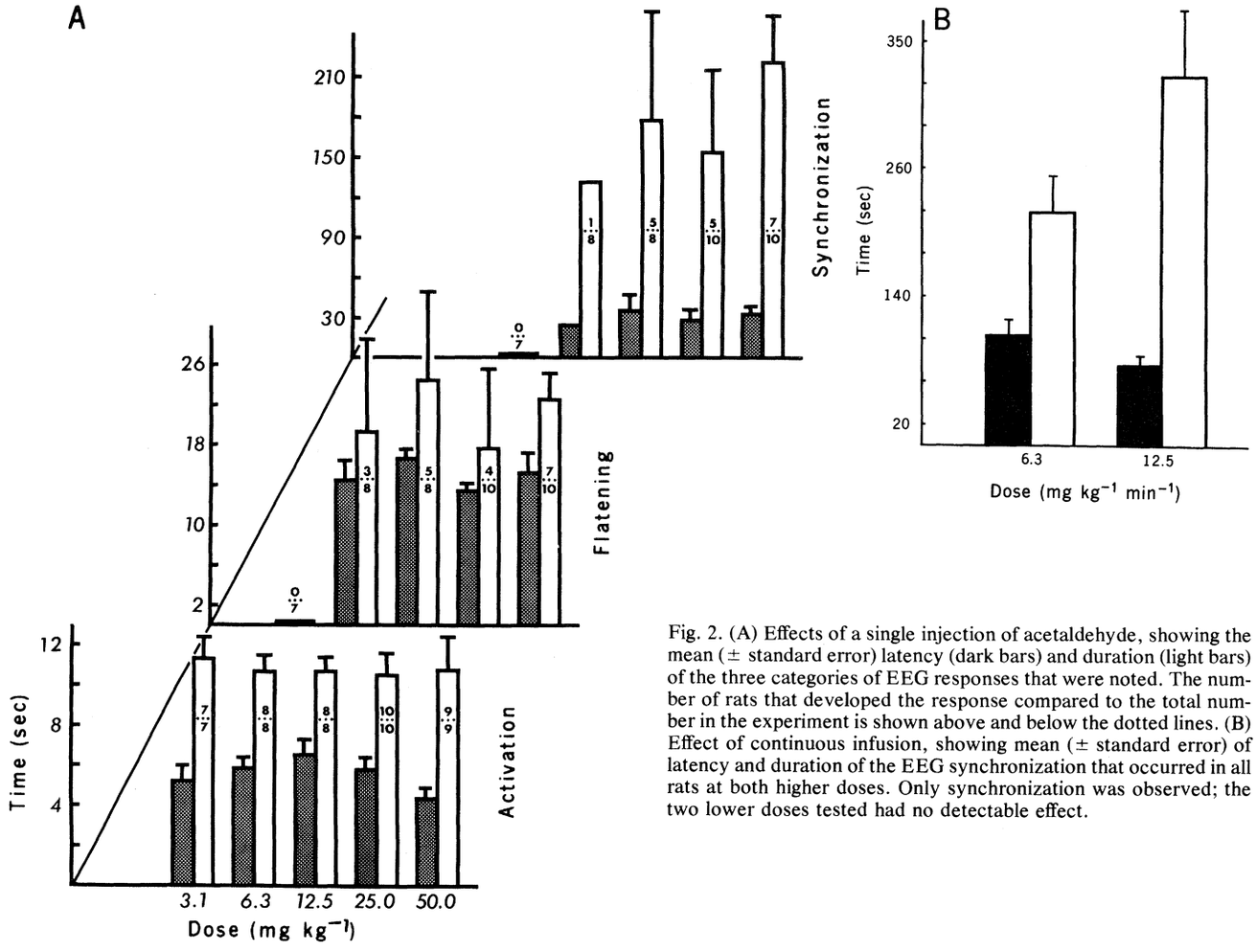

Fig. 2. (A) Effects of a single injection of acetaldehyde, showing the mean (± standard error) latency (dark bars) and duration (light bars) of the three categories of EEG responses that were noted. The number of rats that developed the response compared to the total number in the experiment is shown above and below the dotted lines. (B) Effect of continuous infusion, showing mean (± standard error) of latency and duration of the EEG synchronization that occurred in all rats at both higher doses. Only synchronization was observed; the two lower doses tested had no detectable effect.

dose of 3.1 mg/kg (P = .05, Cochran's test). The largest dose of acetaldehyde produced shorter latency and greater duration of deactivation. In no case was activation observed (Fig. 2).

Saline infusion induced a mild tachycardia (about a 5 percent increase), whereas all doses of acetaldehyde caused a more distinct bradycardia. The response was mild and of approximately similar magnitude for the two low doses of acetaldehyde, but was very pronounced at the largest acetaldehyde dose which produced a maximum depression in heart rate of 16 percent. Electrocardiogram abnormalities were rarely observed in the infusion study and occurred with an approximately equal frequency in the saline and acetaldehyde infusions.

Infusion of the lowest acetaldehyde dose in two rats produced blood acetaldehyde concentrations of 5 and 6 ng/μl, respectively, after 2 minutes of infusion. The concentrations increased to 8.5 and 11.5 ng/μl, respectively, at 4 minutes and returned to near zero within 2 minutes after the end of the infusion. The blood concentrations produced by the larger dose in two rats were much higher, being 51 and 52 ng/μl, respectively, after 2 minutes and 75 and 82 ng/μl, respectively, after 4 minutes; levels gradually declined to 39 and 52 ng/μl 6 minutes after the infusion.

The possibility that peripheral influences mediated the results of the infusion experiments seems to be ruled out by the essentially similar results obtained with transected and adrenalectomized rats (Table 1). Again, infusion with saline in those rats was without effect.

Our results are especially pertinent to the present controversies over the possible role of acetaldehyde in ethanol intoxication. We believe that the data provide convincing evidence that single injections of acetaldehyde are inappropriate for evaluating such questions and that effects which occur under such conditions are more likely to reflect acetaldehyde toxicity, mediated in part, perhaps, by catecholamines.

Continuous infusion can simulate the natural continuous production of acetaldehyde after ethanol administration, and our results show that at least the stage of EEG activation after ethanol administration is not readily explained by a slow release of low levels of acetaldehyde. The role of acetaldehyde in mediating ethanol-induced synchronization should perhaps be more carefully considered. Administration of intoxicating doses of ethanol to rats was reported to produce blood concentrations of acetaldehyde of 0.88 to 6.82 ng/μl (*18*), which is definitely below the peak levels we observed during infusion of the lowest dose, a dose that failed to cause any synchronization in any rat. The highest infusion dose produced a profound synchronization in all six animals tested, but the maximum concentrations of acetaldehyde in the blood of these animals was approximately 20 times higher than that which would result from an intoxicating dose of ethanol. Thus, it appears that acetaldehyde alone, in concentrations that are similar to those found in an ethanol-intoxicated rat, is not adequate to produce ethanol-like EEG synchronization.

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10. The cortical electrodes were fabricated from 30-gauge Formvar-insulated, nichrome wire. The two frontal electrodes were placed 1 mm anterior to bregma, and 2 mm on either side of the midline. The parietal electrodes were placed bilaterally 3 mm posterior to bregma and 3 mm from the midline.
11. Local anesthesia of wound margins was produced by means of lidocaine hydrochloride. Neuromuscular blockage was induced by Flaxedil (0.01 mg/kg).
12. Spontaneous EEG was recorded on a Grass model 78 polygraph with 7P511 amplifiers. Some data were stored on magnetic tape with an Amplex model PR 500 recorder-reproducer.
13. The EEG signals were monitored at three different passbands to facilitate visual detection of drug-induced changes. The three passbands used were 0.3 to 90 Hz, 10 to 90 Hz, and 0.3 to 30 Hz.
14. W. R. Klemm, *Animal Electroencephalography* (Academic Press, New York, 1969).
15. For each animal, heart rate was sampled at successive 10-second intervals. Sampling began 100 seconds before the beginning of injections or infusions and ended 150 seconds after the start of injections or the end of infusions. For each infusion or injection dose the mean heart rate for each 10-second interval was obtained by averaging across animals. The mean heart rate before injection for all animals at a given dose was determined by averaging the ten means obtained before injection (or before infusion). The maximum change in heart rate is expressed as a deviation from this mean heart rate before injection.
16. In all experiments, the order of administration of different acetaldehyde doses (and saline) was randomized. All infusions lasted 240 seconds.
17. C. J. P. Eriksson, H. W. Sippell, O. A. Forsander, in *The Role of Acetaldehyde in the Actions of Ethanol*, K. O. Lindros and C. J. P. Eriksson, Eds. (Kauppakirjapaino, Helsinki, 1975), pp. 9-18; G. Duritz and E. B. Truitt, *Q. J. Stud. Alcohol* **25**, 498 (1964). Blood samples were prepared for analysis by adding 100 μl of arterial blood to 100 μl of a solution containing $HClO_4$ (0.6*M*), thiourea (25 m*M*), and *t*-butanol (300 ng/μl) in a 12-ml rubber-capped serum vial. *t*-Butanol was included as an internal standard for ensuring uniformity of injections. A Hewlett-Packard model 5710A gas chromatograph with a 0.4 by 180 cm glass column packed with Chromosorb 101 (Johns-Manville) was used for separation (column temperature, 160°C; injection port 200°C; flame ionization detector 250°C; helium flow rate, 60 ml/min). The samples were equilibrated for at least 30 minutes at room temperature. Portions (500 μl) of headspace gas were injected with a (gastight) 1-ml disposable plastic syringe. Headspace of reference standards (100 ng/μl and 33 ng/μl) was injected repeatedly during each experiment. Injections were discounted if the *t*-butanol amplitude deviated by more than 10 percent from the mean value for the series. Reproducibility of the technique with replicates of standard test solutions was better than 93 percent.
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19. We thank K. H. Dahm for technical advice and supervision regarding the gas chromatographic analyses.

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Stimulation of Human Periaqueductal Gray for Pain Relief Increases Immunoreactive β-Endorphin in Ventricular Fluid

Abstract. *Immunoreactive β-endorphin was measured in the ventricular fluid of six patients with chronic pain. Stimulation of the periaqueductal gray matter in three patients with pain of peripheral origin resulted in significant increases (50 to 300 percent) in the concentration of ventricular immunoreactive β-endorphin. In three other patients suffering deafferentation dysesthesia, stimulation of the posterior limb of the internal capsule did not alter the concentration of this peptide. These results provide evidence of the release of human immunoreactive β-endorphin in vivo and suggest that naloxone-reversible pain relief achieved by stimulation of the periaqueductal gray matter may be in part mediated by the activation of β-endorphin-rich diencephalic areas.*

Since the initial report by Reynolds (*1*) of analgesia being produced in the rat by stimulation of the periaqueductal gray matter, several workers have demonstrated the potent analgesic effects produced by electrical stimulation of discrete areas of the medial diencephalon and brainstem in the cat (*2*), monkey (*3*), and rat (*4*). Hosobuchi *et al.* (*5*) and Richardson and Akil (*6*) have reported that intractable clinical pain states in humans, in addition to normal pain perception, can be blocked by electrical stimulation of the periaqueductal and periventricular gray matter. This pain relief can be totally reversed by the specific opiate antagonist naloxone (*5*, *7*).

Intraventricular administration of human β-endorphin in humans produces a prolonged state of analgesia (*8*). The current study was undertaken to determine whether the analgesia produced by stimulation of the periaqueductal gray matter would be accompanied by increases in the β-endorphin concentrations in ventricular cerebrospinal fluid (CSF).

The specificity of the effect of stimulating the periaqueductal gray matter for the endorphin system was also investigated. For this purpose we selected two groups of patients: group 1, suffering from deafferentation pain; and group 2, whose pain relief is known to be obtained by stimulation of the periaqueductal gray matter or by opiates. In group 1, pain relief is obtained neither by opiates nor by periaqueductal gray matter stimulation, but it can be obtained by electrical stimulation of the internal capsule (*9*). Therefore, should our working hypothesis linking stimulation of periaqueductal gray matter with the endorphin system be correct, treatment of deafferentation pain by internal capsule stimulation should not affect β-endorphin concentrations in ventricular fluid.

Ventricular fluid was collected from six patients undergoing stereotactic implantation of brain electrodes for pain control (Table 1). The three patients in group 1, suffering from deafferentation pain, received electrode implantation in the posterior limb of the internal capsule contralateral to their pain (patients A, B, and C). The patients in group 2, with pain of peripheral origin, and received bilateral implantations of electrodes in the rostral portion of the periaqueductal gray matter (patients D, E, and F). A Leksell stereotactic frame was used on all six patients and the stereotactic coordinates were selected and calculated on the basis of Schaltenbrand and Bailey's human stereotactic atlas (*10*). Coordinates for the posterior limb of the internal capsule were: anteroposterior axis at the line intercepting the anterior and posterior commissure; and ventrodorsal axis 1 mm below the intercommissural line and 15 to 20 mm lateral from the midline of the brain, depending on the portion of the body for which relief of pain is sought, the upper limb being more medial than the lower limb in the "homuncular" representation of the internal capsule. For periaqueductal implantation, the area coordinates were at

Table 1. Clinical summary of the patients (*17*).

Patient	Age	Sex	Etiology of pain	Location of pain
A	47	M	Postcordotomy dysesthesia	Bilateral, lower extremities
B	54	M	Postcordotomy dysesthesia	Right lower extremities
C	63	M	Thalamic syndrome	Right arm and leg
D	51	M	Lumbosacroarachnoiditis	Low back and both legs
E	57	F	Carcinoma of rectum	Abdomen and perineum
F	66	F	Carcinoma of colon	Abdomen

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