# Reversal in central nervous system function during ethanol withdrawal in humans and experimental animals[1]

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ABSTRACT

The ultimate objective in devising animal analogs of physical dependence on ethanol is to obtain meaningful imitations that will have behavioral and biological similarities to human subjects during the ethanol withdrawal syndrome. The natural history of alcoholic disease in human subjects and in experimental animals involves three periods, each characterized on the basis of temporal relationships, pattern of ethanol intake, blood ethanol concentrations, and/or a typical sequence in the onset and decay of the characteristic spectrum and continuum of overt behavioral, neurological, and biological signs and responses. These characteristics are expressions of different functional states of the central nervous system (CNS): *1*) the baseline period or predrinking period reflects normal function of the CNS; *2*) the induction period or drinking period is characterized by overt signs and responses of nonspecific, long-term CNS depression; and *3*) the withdrawal period is characterized by a relatively rapid transition in the CNS function from depression during the prodromal detoxication phase to hyperexcitability observed during the withdrawal syndrome (dependence phase). The rapid transition from overt depression to overt hyperexcitability is a consequence of rapid removal of the drug from the system, and constitutes the basis of the reversal in the CNS function in both humans and experimental animals.—**Majchrowicz, E.** Reversal in central nervous system function during ethanol withdrawal in humans and experimental animals. *Federation Proc.* 40: 2065–2072; 1981.

The control and prevention of alcohol abuse and alcoholism could be accomplished relatively quickly simply by eliminating the consumption of alcoholic beverages. This proposition is, however, of only theoretical consideration because victims of alcoholism can seldom be persuaded to avoid ethanol, despite its tragic results. Therefore, corrective measures have to be undertaken by a variety of approaches. Regardless of the method of treatment, a prior understanding of the biochemical and behavioral correlates of ethanol intoxication, tolerance, and addiction is of primary importance. Although a number of clinical, behavioral, and pharmacological parameters can be studied relatively easily in human subjects, the neurobiological correlates are difficult to investigate in man. Therefore, the availability of an appropriate animal analog of ethanol dependence constitutes an essential prerequisite for the successful study of the primary mechanisms underlying addiction to ethanol (2, 4, 6, 7, 11, 20, 21, 22, 32, 38, 39, 41).

The ultimate objective of devising an animal analog of physical dependence on ethanol is to obtain a meaningful substitute that will resemble its human counterpart, both behaviorally and biologically. To achieve this goal it is essential to use similar methods and patterns of ethanol administration and to identify in the animal signs and responses of the ethanol withdrawal syndrome analogous to those found in humans. The value of these animal analogs depends largely on the similarities between the withdrawal syndromes in humans and animals (20, 22, 24, 25, 38).

## NATURAL HISTORY OF ETHANOL DEPENDENCY

The administration of a single dose of ethanol results in nonspecific general depression of the central nervous system (CNS) and subsequent behavioral changes, the severity of which is directly related to the amount of ethanol consumed and to the subsequent blood ethanol concentration (BEC) (9, 28, 29). Upon clearance of ethanol from the blood, the organism and the CNS may return to an earlier, presumably normal, homeostatic state. The hangover after a single episode of ethanol abuse, however, suggests longer lasting central nervous system changes, which are not clearly understood at present.

Repeated doses of ethanol during long-term drinking prolong the nonspecific depression of the CNS and induce longer-lasting changes in body and brain homeostasis. Upon termination of long-term drinking, the CNS must function temporarily at a new homeostasis, until the situation gradually returns to the predrinking state. This readaptation, when long-

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term drinking is terminated and ethanol eliminated from the system, constitutes the basis of the ethanol withdrawal syndrome (2, 4–6, 13, 19–21, 31, 32, 38, 41). The rapid transition from long-term depression of the CNS to the withdrawal hyperexcitability represents a reversal in CNS function during the postwithdrawal period and is well recognized clinically and behaviorally in humans under emergency and experimental ward conditions and in experimental animals as the ethanol withdrawal syndrome (2, 4, 6, 10, 19, 21, 31, 33, 38, 41).

To clarify these issues, it is necessary to recognize in detail the natural history of alcoholic disease in humans as well as the biologic history of the induction of ethanol dependency in experimental animals. The term natural history as used here signifies the chronological sequence of past and present events or temporal periods in the life of a subject that lead to, sustain, and follow the alcoholic disease in humans or physical dependence upon ethanol in experimental animals. The events and/or temporal periods are identified by a spectrum and continuum of characteristic signs and symptoms in human subjects, or signs and responses (reactions) in experimental animals. Briefly, there are a number of consecutive periods that can be clearly distinguished on the basis of temporal relationships, blood ethanol concentration, and a characteristic sequence in the onset and disappearance of corresponding signs, symptoms, and responses in humans and experimental animals (4, 6, 12, 19, 21, 22, 31, 33, 36, 38). These clearly defined periods reflect the functional state of the CNS at that particular point in time. In addition, by clearly defining and recognizing these time periods in the history of ethanol dependency, the discrepancies in the results and interpretations among different laboratories might be narrowed or even eliminated.

Figures 1 and 2 show the three basic periods in the natural history of alcohol dependency in humans and animals under experimental conditions respectively:

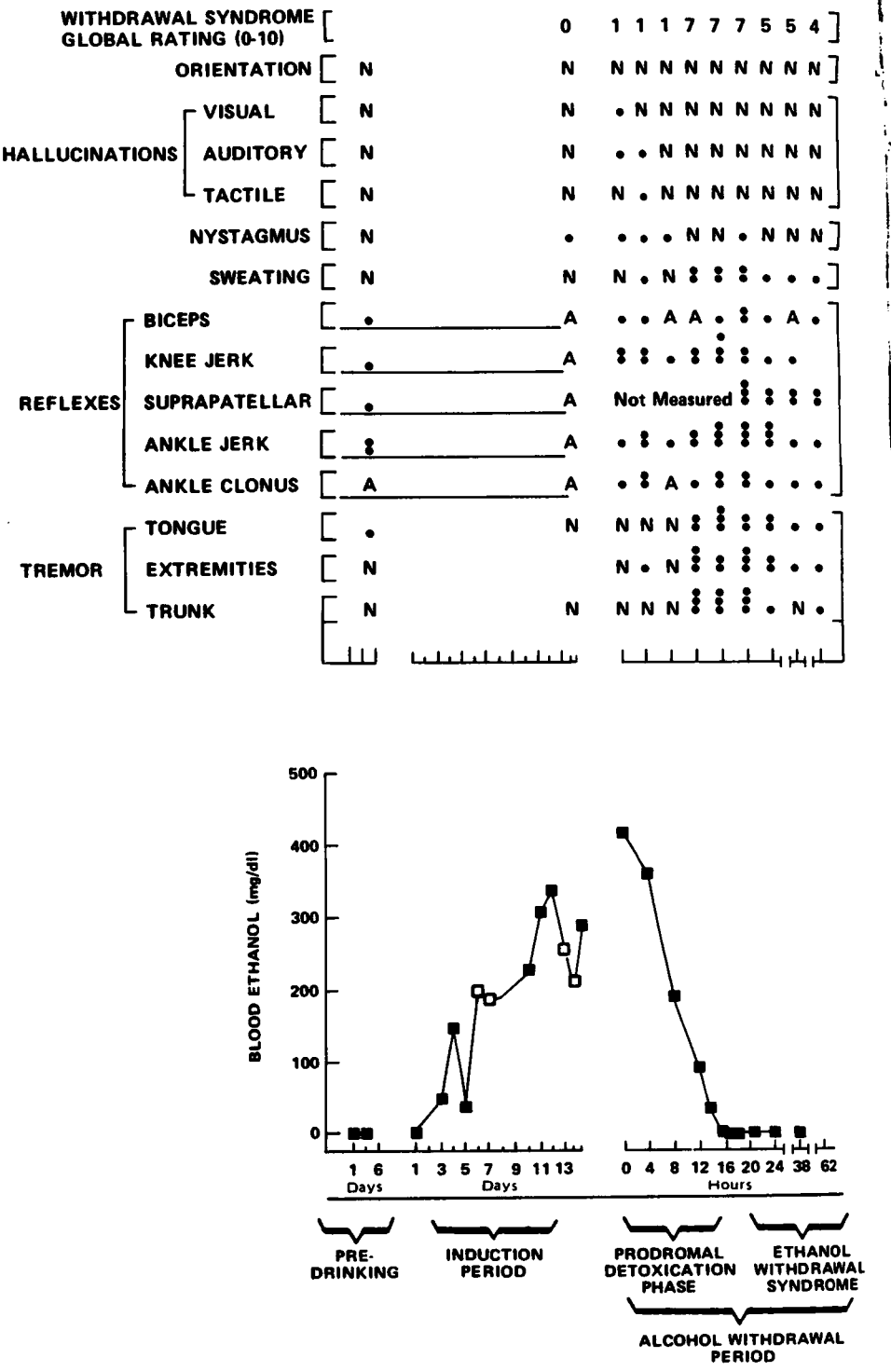



**Figure 1.** The natural history of ethanol dependency during a 15-day experimental drinking period (induction period) in male alcoholic subjects. Blood ethanol concentrations (BEC) were determined on mornings of the induction period and at the designated time intervals during the ethanol withdrawal period. BEC were determined by automated gas chromatography (full squares) or by Breathalyser (open squares). The severity of the withdrawal signs and symptoms was assessed subjectively on a scale of 3 to 1 (black circles). A = absent; N = normal. For procedural details see literature cited in the text (19, 26, 27, 31, 38). Slightly modified and reproduced from ref. 22 with permission.

*1) The Predrinking Period* may be pharmacologically defined as the *baseline period*, during which both human and animal subjects are acclimated to research ward conditions and during which time a number of control measurements may be taken. The predrinking period in chronic alcoholics may be limited to the first 15 to 20 years of life. In such case, it might be difficult to establish a genuine baseline in experimental subjects.

2) Then follows the *(Experimental) Drinking Period*, pharmacologically de-

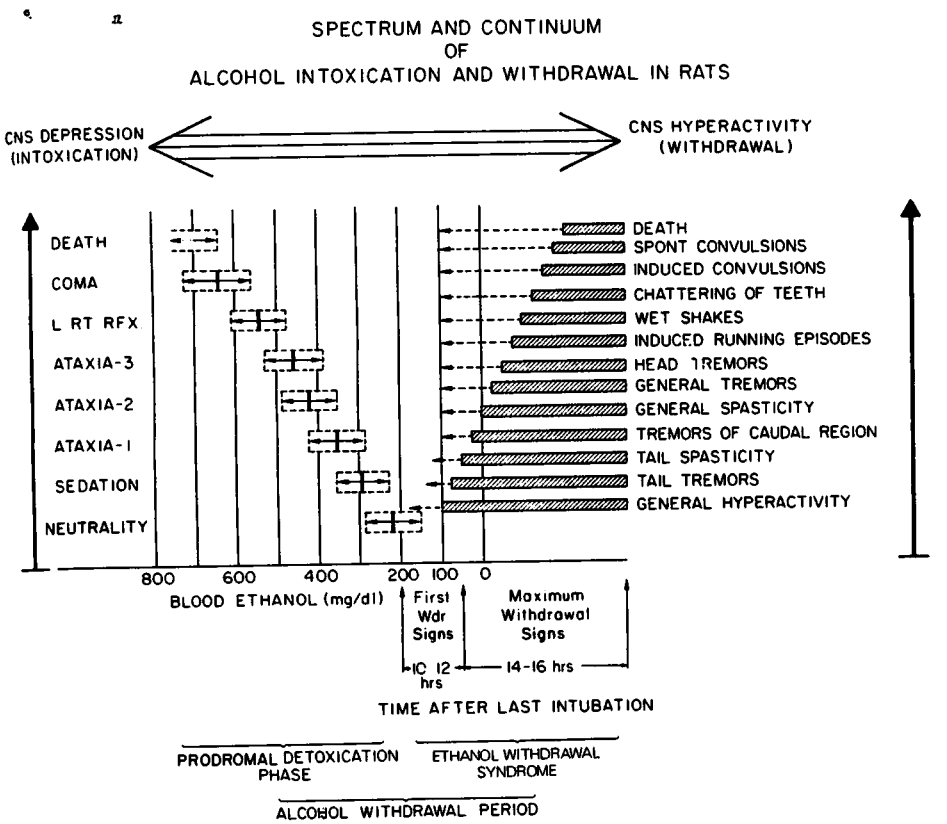



**Figure 2.** Spectrum and continuum of signs and responses observed during the ethanol withdrawal period in rats. Two characteristic phases of the withdrawal period are identified by specific spectra of signs and responses, which are expressions of the functional state of the central nervous system during that particular time interval. Reproduced with permission from E. Majchrowicz, *Psychopharmacologia*, 43: 245; 1975.

fined as the *induction period*, during which the subjects consume or are given ethanol in a variety of alcoholic beverages. The duration of this period and the amount of the ethanol consumed can be controlled by the experimenter according to his requirements.

*3*) The *Withdrawal Period* usually begins with the last drink or the last dose of ethanol given at the termination of the induction period. During the withdrawal period, there are two distinctive phases, one of which is characterized by the disappearance of signs and symptoms of CNS depression, and the other, by the onset of the signs and symptoms of the withdrawal syndrome in humans (or signs and reactions in experimental animals). Since in this paper we discuss the biobehavioral characteristics of the ethanol withdrawal syndrome in human subjects and physical dependence upon ethanol in experimental animals, it should be noted that the terms, signs, and symptoms are ordinarily used with reference to human subjects only, whereas the terms, signs, and responses (and/or reactions) are restricted to animals. The term "symptom" denotes subjective events or conditions that are reported or communicated by the patient to the investigator, which are usually not observed directly by the investigator; e.g., mental activity, sensory functions, hallucinations, delusions, orientation in time or space, etc. Signs and responses (and/or reactions) denote physical changes and events that can be objectively observed, studied, and measured or rated by the investigator; e.g., tremors of hands, limbs, body, tail or head, body rigidity, spontaneous and induced convulsive seizures, hyperreflexia, muscle fasciculation, chattering of teeth, wet shakes, hyperactivity, stereotype body movement, induced running episodes, etc.

Furthermore, the signs and responses characterizing the two phases are expressions of the variety of neurological, behavioral, clinical, biochemical, and pharmacological changes induced in the body and particularly in the central and peripheral nervous systems. The two phases are: *1*) the prodromal detoxication phase and *2*) the ethanol withdrawal syndrome.

## CHANGES IN FUNCTIONAL STATE OF THE CNS DURING INTOXICATION AND WITHDRAWAL

These three periods in the natural history of alcoholic disease reflect three different functional states of the CNS. It is assumed that during the predrinking period the CNS is in a normal condition, and this period usually serves as the reference or control state. During the induction period, the CNS is nonspecifically depressed by the continuous intake of ethanol and the subsequent high blood ethanol concentration (BEC). This state of depression is reflected by overt signs of intoxication and by the concomitant "see-saw" pattern of the BEC curve (Fig. 1; refs. 21, 23). The first phase of the withdrawal period, i.e., *the prodromal detoxication phase*, is essentially a continuation of the CNS depression initiated during the induction period. *The withdrawal syndrome* is the second phase of the withdrawal period when physical dependence is overtly expressed as the withdrawal signs, reactions, and/or symptoms. Between the two is a short period of "pseudo-normalcy," followed by the onset of the withdrawal signs and reactions reflecting the hyperexcitability of the CNS. Although dependency is induced at some time during ethanol intoxication, an accurate determination of the time of its acquisition is difficult for a number of reasons. It is apparent that the dependency is concealed during the latter part of the induction period and during the prodromal detoxication phase, when it exists in a state of latency. The potential for the expression of the hyperexcitability in the CNS is antagonized by the presence of ethanol. The dependency cannot be revealed until ethanol consumption is terminated and the BEC subsequently declines to a certain threshold level, when the dependency is manifested by the characteristic signs, responses, and/or symptoms of withdrawal hyperexcitability. The onset of withdrawal signs and responses at relatively high BEC marks the initiation of the withdrawal syndrome (dependence phase). Following recovery from the withdrawal reactions, the organism

and the brain may eventually return to the predrinking, or baseline, state.

## INDUCTION PERIOD

### Induction period in humans

The natural history of the induction, onset, and duration of ethanol dependency in rats has many similarities and analogies to its human counterpart (2, 6, 11, 19, 20, 22, 24, 31, 38). The sequence of consecutive periods outlined above can be observed in both humans and experimental animals under a variety of conditions. One requirement for induction of the withdrawal syndrome in humans is a state of continuous CNS depression for a relatively short period of time, which may be achieved by frequent consumption of small doses of a variety of alcoholic beverages. In the experimental ward continuous depression was achieved by repeated consumption of a variety of beverage ethanol preparations in multiple small doses up to 32 one-ounce fractions per day for up to 15 days (19, 26, 27, 31). The subjects were under continuous nursing staff observations and were not allowed to attain extremes in either intoxication or withdrawal reactions (26, 27, 31).

Although in chronic alcoholism the state of mild to moderate depression is sustained for long periods of time, it is usually an acute binge of excessive drinking that precipitates withdrawal reactions. Such a binge is comparable to the induction period under experimental conditions. When, for a variety of reasons, a person stops drinking, the binge is interrupted, resulting in the development of typical withdrawal signs and symptoms that correspond quite closely to those seen under experimental ward conditions. In order to alleviate the onset of the withdrawal syndrome, recognized as morning tremulousness, the alcoholic will occasionally take more alcohol, but in severe cases, he may be rushed to the hospital where he develops a full-blown withdrawal syndrome (31, 38, 39). The difference between uncontrolled and experimental drinking is that during an alcohol binge, the person concerned consumes alcohol without any restrictions; consequently, the withdrawal syndrome may be much more severe than under experimental conditions where drinking is controlled.

### Induction period in animals

The induction period in animals is characterized, as in humans, by a relatively short-term, nonspecific CNS depression. Although a number of criteria suggested certain requirements for an animal analog of physical dependence upon ethanol (2, 7, 20, 30), some of these requirements have been difficult or impossible to implement (20, 30). To achieve a successful induction period in animals, researchers usually do not rely on voluntary consumption, but find it necessary to administer ethanol in a variety of ways (2, 4, 5, 7, 12, 20, 21, 30, 32, 33, 41). In order to simulate the pattern of alcohol consumption in humans, ethanol was administered to rats by intragastric intubation (using a baby feeding tube) at the rate of 8 to 12 g/kg per day in 6 to 9 fractional doses as a 20% (wt/vol) solution for 4 days (3, 14, 15, 17, 21, 23, 35, 40).

After the first (priming) dose, subsequent dose levels were determined for each animal on the basis of the severity of intoxication assessed prior to intubation. Although time sufficient to induce dependence may vary, it was found that 4 days was adequate for the development of a full spectrum of withdrawal signs and reactions in rats and mice (2–4, 6, 10, 14, 15, 17, 21, 23, 33, 35, 40, 41). It should also be noted that since the maximum rate of ethanol metabolism in the rat is about 7 to 9 g/kg per day, maximum daily doses cannot greatly exceed this amount. If the rat receives more than the maximum, the excess is carried over to the following day; consequently, the total daily dose must be reduced to avoid mortality.

## ETHANOL WITHDRAWAL PERIOD

### Prodromal detoxication phase

In both humans and experimental animals, the CNS depression initiated during the induction period continues after the last ethanol dose, i.e., after the initiation of the conventional withdrawal period (Figs. 1 and 2), and in both the withdrawal period has two clearly distinguishable phases: the *prodromal detoxication* phase and the *withdrawal syndrome* (the *dependence phase*). In both humans and experimental animals, the prodromal detoxication phase is characterized by the rectilinear rate of ethanol clearance from the blood, with concurrent lessening of the CNS depression, and can be observed as a gradual sobering of the patients or animals. The sobering is essentially the reversal of the gradual intoxication process, as BEC increases after the initiation of drinking episode (8, 9, 28, 29). To identify the corresponding signs of CNS depression in the human and animal subjects, reference is made to Table 1, which shows the relationship between changes in the symptoms of mood and behavior, signs of motor incoordination, and their close relation to changes in the concurrent BEC.

Although basically none of the symptoms of mood and behavior observed in human subjects (e.g., changes in mood, impaired mental activity and sensory functions, Fig. 1, Table 1) can be identified in animals with certainty, a number of signs of motor incoordination have their equivalent in the rat: degrees of ataxia, loss of righting reflex, vital signs (e.g., respiration, heart rate, eye blink reflex, swallowing reflex), stupor, and coma (2, 4, 7–11, 13, 20, 23, 28–30, 32, 33, 38). As the BEC declines to approximately 200 to 100 mg/dl, with consequent diminution of CNS depression, there is a short-lived period of pseudonormalcy, during which patients attempt eating and drinking, washing, shaving, and resuming social contact. An analogous behavior stage is observed in experimental animals. During this short-lived stage of neutrality (Fig. 2) the animals start grooming, attempt to eat, drink, and explore the cages. As with human subjects, the brief neutrality is followed by gradual onset of the withdrawal reactions. Both in humans and in experimental animals, the neutrality stage constitutes the transition point in CNS function from depression to hyperexcitability.

### Withdrawal syndrome in humans

The onset of the withdrawal syndrome in humans occurs at relatively high BECs (19, 31, 38; Fig. 1); at approximately 200 to 100 mg/dl there is a gradual onset of signs and symptoms. Signs include reflex and motor changes typically represented by tremors of hands, tongue, body, and head. Tremors are universally found in all species of subjects undergoing pro-

TABLE 1. Relationship between the signs and symptoms of intoxication and the concurrent blood ethanol concentrations in human subjects

| Blood level of ethyl alcohol (mg/dl) | Effect |
|---|---|
| 20–99 | A. *Impaired sensory function*<br>1. Reduced visual acuity (flicker-fusion test)<br>2. Decreased sense of smell and taste<br>3. Elevated threshold for pain<br>(a) Decreased sensitivity of cornea of eye<br>(b) Decreased sensitivity to local heating of skin<br>B. *Muscular incoordination*<br>1. Spontaneous and induced nystagmus<br>2. Decreased steadiness while standing (Romberg test)<br>3. Impaired performance on tests of skill (Ring test, Finger-to-finger test, target practice, typing)<br>4. Slight impairment of ability to drive an automobile<br>C. *Changes in mood, personality, and behavior*<br>1. Dizziness<br>2. Reduced sense of fatigue<br>3. Mild euphoria<br>4. Self-satisfaction<br>5. Release of inhibitions<br>6. Loud, profuse speech<br>D. *Impaired mental activity*<br>1. Subtraction test<br>2. Reading comprehension tests |
| 100–199 | A. Staggering gait<br>B. Marked impairment on mental tests<br>C. Marked impairment of driving ability<br>D. Lengthened reaction time |
| 200–299 | A. Nausea and vomiting<br>B. Diplopia<br>C. Marked ataxia<br>D. Extreme clumsiness |
| 300–399 | A. Hypothermia, cold clammy skin<br>B. Loss of ability to speak<br>C. Amnesia<br>D. Anesthesia<br>E. Heavy breathing |

The table was published under the title "Blood levels of ethyl alcohol in man and effects on sensation, muscular coordination, performance, behavior, skill and judgment" in "Toxicology of single doses of ethyl alcohol" by Harriet M. Maling (28). Reproduced with permission from the author and the publisher. Also consult Goldberg (8, 9), Mardones (29), and Victor and Adams (38).

nounced withdrawal reactions and therefore, the spectrum of tremors is a valuable set of signs to characterize the withdrawal reactions in animals. Essentially none of the symptoms of mood and affect, changes in mental activity, or sensory functions can be identified in animals. Consequently, the symptoms of withdrawal found in humans have relatively little application in animal situations.

## Convulsive seizures

Although convulsive seizures constitute one of the most dramatic and severe signs of the withdrawal reactions, they are not often seen under experimental ward conditions. Basically, the alcohol withdrawal syndrome in chronic alcoholics very much resembles that observed under experimental ward conditions. The natural history of the withdrawal syndrome has been discussed extensively by Victor et al. (36–39), Isbell et al. (19), Mendelson et al. (31), and others, and a number of their papers constitute classics in this area of alcohol research. However, there are a number of significant differences between natural and experimental conditions:

*1*) One difference is that the conventional initiation of the withdrawal period cannot be accurately established in an intoxicated person brought to the emergency ward for treatment. Therefore, the potential onset of the withdrawal reactions must be assessed by determining the BEC and by frequent, systematic observation of the patient. As mentioned above, the withdrawal reactions start to appear when the BEC attains a certain threshold value.

*2*) The proportion of convulsive seizures in alcoholics brought for emergency treatment is considerably higher than in those under experimental ward conditions. As shown in Fig. 3, the convulsive seizures are all spontaneous. Induction of seizures in any clinical ward is not allowed. Most seizures occur within 24 h after admission to the emergency ward (37, 39) and in this respect a close similarity exists between humans and rats (22, 24), where most if not all spontaneous convulsions occur within 24 h after the initiation of the withdrawal period (21, 23).

*3*) Excepting perhaps the studies by Isbell et al. (19) in the 1950's in Lexington, I am not aware of any experiments in which human subjects were allowed to develop the full spectrum of withdrawal signs and symptoms including convulsive seizures and delirium tremens. If the latter signs occurred under experimental ward conditions, they were usually accidental. Delirium tremens usually occurs in hospitals after patients are brought for emergency treatment. The mortality from delirium tremens once was about 15% of the population brought for emergency treatment (36, 37, 39), but since the introduction of effective symptomatic treatment with drugs, the mortality rate has been reduced nearly to zero. To date, nothing analogous to delirium tremens has been reported in experimental animals.

## Assessing withdrawal reactions in human subjects

Evaluation of the severity of the withdrawal reactions in humans in both experimental and emergency ward situations is conducted under conditions that preclude either the stimulation or exacerbation of the withdrawal reaction. Thus all noise, loud music, and glaring lights are elim-

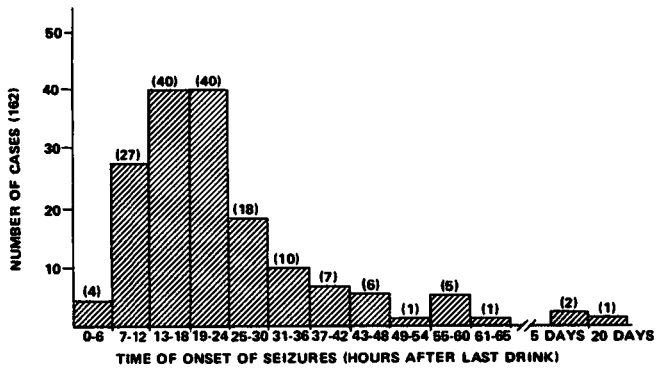



**Fig. 3.** Temporal relationship of convulsive seizures during ethanol withdrawal syndrome in male alcoholics. Reproduced from M. Victor and C. Brausch, *Epilepsia*, 8. 1; 1967 with permission.

inated. Each of these stimulants may not only exacerbate the withdrawal reactions, but potentially may precipitate convulsive seizures, which should be avoided under all circumstances.

The rating of the withdrawal reactions in humans is based on a set of subjective and objective measures, some of which are shown in Fig. 1 (19, 31, 38). As indicated above, convulsive seizures constitute one of the most severe signs of CNS hyperexcitability; they are not often seen under experimental ward conditions and are not used as general signs for rating the severity of the withdrawal syndrome.

## Withdrawal syndrome in rats

As in humans, the onset of ethanol withdrawal syndrome in rats occurs at relatively high blood ethanol concentrations. Usually, the gradual appearance and intensification of CNS hyperactivity is reflected in the withdrawal signs and reactions. The reversal in CNS function—from severe depression to severe hyperexcitability—occurs in a relatively short time period (Fig. 2). This onset of withdrawal reactions at relatively high BECs and in a short time period was probably one cause of the confusion mentioned by Victor, who wrote (36) that "The literature has been characterized by outstanding confusion, inexactness and contradictions; the major shortcomings of which were that no clear distinction has been made between the problems of acute alcoholic intoxication, alcohol addiction, mild withdrawal signs and symptoms, and delirium tremens."

## Convulsive seizures and scoring of withdrawal reactions in rats

Although spontaneous convulsions occur only in about 20 to 30% of rats tested for withdrawal reactions, the induction of convulsions by audiogenic means succeeds in about 30 to 40% (E. Majchrowicz, E. F. Williams and P. Skolnick, unpublished results). This is quite different from the situation in mice, in which all tested are susceptible to induced convulsions, and this sign has been used as the sole marker for scoring the severity of withdrawal (10, 13).

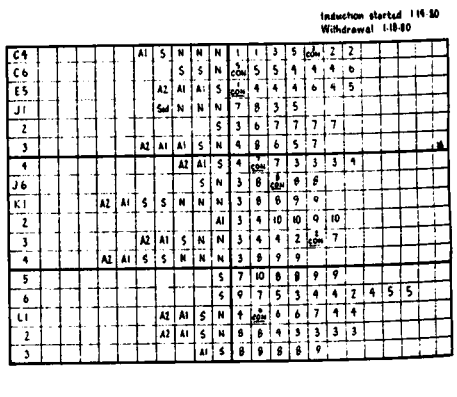
Another important distinction between spontaneous and induced convulsions is the mortality rate. Usually about 90 to 95% of rats undergoing spontaneous convulsions survive the event (21). However, the audiogenically induced convulsions and the intimately associated running episodes usually terminate in mortality. The animals do not survive longer than about 10 to 20 minutes after the induction of convulsions (E. Majchrowicz, E. F. Williams, and P. Skolnick, unpublished observations).

Therefore, when observing and rating the animals, we adopted conditions and criteria similar to those applied on the clinical ward for evaluating withdrawal reactions in human subjects. Thus, all noise was eliminated; the animals were handled carefully to avoid exacerbating the withdrawal reactions and precipitating convulsive seizures. The observations were carried out in nonglaring light. Jingling of keys, metal cage covers, and glassware was avoided.

Although we usually observed the entire spectrum of signs and reactions shown in Fig. 2, for the purpose of scoring the withdrawal syndrome severity we found the utilization of tremors of tail, caudal region, head, and total body as the most reproducible and reliable procedure (14, 25). This choice of signs is based on the observation that tremors constitute the most characteristic and prevalent sign of withdrawal in both human subjects and rats. Second, the other signs and reactions listed in Fig. 2 are difficult to quantitate on a differential scale and, consequently, are recorded only as either present or absent. Observing other signs of the spectrum assists in identifying the presence of the withdrawal syndrome. Third, since spontaneous seizures occur in only about 25% of withdrawing rats and 15% of humans, this sign obviously cannot be utilized for nonconvulsing animals. Animals displaying seizures constitute a separate, behaviorally distinct group.

The withdrawal period by definition begins with the last dose of ethanol, given usually the evening preceding withdrawal day. Starting at

**Figure 4.** A sample withdrawal sheet recording the spectrum of behavioral changes during the withdrawal period from 12 through 24 h after the last dose of ethanol. The first observation for each rat was recorded at the 12th hour after the initiation of the withdrawal period. Symbols: A2 = ataxia 2; A1 = ataxia 1; S = sedation; N = neutrality. Other signs of intoxication, i.e., coma, loss of righting reflex, ataxia—3 (21–23) were not observed during this rating period. The withdrawal score was based on a subjective evaluation of the tremors of tail, caudal region, trunk, and head. Tremors were rated on a scale of 3 to 1. The numbers represent the sum of individual scores assessed at 1-h intervals. All other signs and responses shown in Fig. 2 are unamenable for subjective quantitation. For more details of the rating system, see refs. 21–23, 25.



7:00 AM, the rats are rated systematically at hourly intervals for signs of either intoxication or withdrawal (Fig. 4; 21, 23, 25). This system of hourly ratings is absolutely essential to obtain an animal with well-defined behavioral characteristics that could be definitively assigned to a specific phase of withdrawal period, i.e., the preparation of a behaviorally and functionally homogeneous sample. Both the determination of BEC and the observation of a variety of signs and reactions ensures assignment of the animal to an appropriate phase that reflects the concurrent status of the CNS functional state. In Fig. 4, the scoring is arranged around neutrality as a central point of the withdrawal period. The animals to the left of the neutrality line are still in a functionally depressed state, i.e., they are intoxicated. On the right, the scoring indicates that the animals have crossed the neutrality demarcation line and are undergoing the overt withdrawal syndrome. It should be added that at the crossover line the BEC is around 100 to 200 mg/dl. To ensure that all blood ethanol has been cleared from a representative group, and to eliminate a possible interaction between ethanol and a test drug, the animals are allowed to withdraw for 4 to 7 hours before their decapitation and use for neurochemical or pharmacological studies (Fig. 4; 25). It is evident that an arbitrary assignment of animals into any category merely on the basis of time elapsed after the initiation of the withdrawal period—i.e., without the behavioral observations and without the determination of BEC—precludes the selection of a homogeneous sample. This is one of the most likely causes of discrepant results from different laboratories. The basic need for an accurate characterization of the behavioral and functional state of the animals at the time of testing has been addressed in a number of publications (1, 2, 4, 7, 13, 20, 21, 25, 30, 37). A number of studies have demonstrated the critical importance of accurately identifying the behavioral phase of the withdrawal period in relation to changes in the concentrations of a number of neurochemical components. For example, $K^+$-stimulated release of dopamine in corpus striatum was substantially elevated when measurements were made before the onset of the withdrawal syndrome at the time when rats displayed marked signs of intoxication and their BEC was still high. However, dopamine release was substantially reduced during the overt withdrawal syndrome when animals developed severe signs and reactions of the withdrawal syndrome and their BEC was zero (3). Similar biphasic response, both with respect to BECs and the phase of the withdrawal period, was found in norephinephrine turnover (16, 34) and acetylcholine content (15). The significant changes in either the turnover rates or the steady-state concentrations of biogenic amines coincides quite accurately with the concurrent changes in behavior during the relatively short period of transition when the CNS function moves from depression (prodromal detoxication phase) to hyperexcitability (ethanol withdrawal syndrome). Whether these changes are the causes or effects of dependency on ethanol remains to be established.

Although the methods discussed in this review for comparative evaluations of physical dependence upon ethanol and for the evaluation of the reversal in the CNS function in rats may not satisfy all needs for solving the problems concerning the addiction to ethanol, their major characteristics are accurate definitions of terms, reproducibility, rapidity, and analogy to human counterparts (1, 7, 11, 14, 15, 21, 23, 25, 35, 40). FC

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