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Operant Behavior and the Measurement of Motor Dysfunction

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Motor disturbances dominate the neurobehavioral effects reported after chemical exposure (1,2). These motor disturbances include incoordination or clumsiness, tremor, ataxia, weakness, and postural disturbances and they all appear in behavior. Sometimes the disturbances are overt and incontrovertible; at other times they can be subtle and detectable only by the one experiencing them, or on careful examination (3,4).

These disturbances generally reflect motor function, and our understanding of them can be furthered by following a strategy similar to that for sensory function. That is, a detailed characterization of the structure and function of the system as well as its expression in behavior is required. The strategic link between the general approaches to the study of motor and sensory function has been identified by Shepard:

> These similarities [between motor and sensory systems] mean that we can approach the study of motor systems using the same logical approach to basic mechanisms that was applied to sensory systems. The basic mechanisms operate at similar levels: the peripheral *organs*, the *neural circuits*, and the *behavior* of the whole organism. (5 p 358)

Although the general strategies taken for studying sensory and motor function may be similar, the tactics reflect the special problems raised by the execution of behavior. These problems differ from those presented in the study of how stimuli influence behavior. Nevertheless, in both domains the degree to which dysfunction can be predicted, characterized, and prevented depends in part on our ability to identify functional impairment in the behavior of intact animals. The experimental analysis of behavior and our understanding of schedule-controlled operant behavior (SCOB) provide techniques for controlling behavior and a theoretical framework within which the behavior of intact organisms can be understood.

This chapter examines tactics for applying operant behavior to the study of motor dysfunction in nonhuman subjects. Although operant behavior is certainly not the only place that motor effects appear, it has some distinct advantages. These advantages include an ability to manipulate reinforcement contingencies to synthesize a response that can be studied, the presence of a large body of data describing indirect behavioral effects of reinforcement schedules that often are motor in nature, well-understood research-strategies that reveal motor effects sufficiently reliably for risk assessment, and the presence of a conceptual framework for identifying mechanisms either at the level of behavior or at the level of the organs and neural circuits underlying behavior.

This chapter addresses these issues. First, SCOB is defined and described, with an emphasis on how it has contributed to advanced strategies for studying neurotoxicity and, especially, motor function. Schedules of reinforcement have both direct and indirect effects on behavior. Indirect effects are not explicitly specified but appear consistently in behavior. To show how indirect effects can be incorporated into other advanced applications, an example is drawn from an examination of manganese's effects on response durations. Direct effects are explicitly defined in the schedule and can be used to synthesize behavior suitable for the study of a specific motor end point. Many examples could be provided: tracking accuracy, movement times, postural stability, and positioning to name a few. The synthesis of a positioning response used to examine tremor exemplifies this tactic.

Both examples illustrate research designs appropriate to advanced study of toxic effects in a small number of subjects. Some advantages achieved by applying operant techniques are examined by showing that the range of potentially toxic doses can be narrowed by tailoring the response to the question. In addition, sensitivity is improved over that provided by observational techniques. The chapter concludes with a brief discussion of the different ways in which mechanisms are incorporated into the characterization of chemical effects on behavior.

COMPONENTS OF OPERANT BEHAVIOR

Operant behavior is fundamentally moldable and plastic. The very definition of an operant as a class of responses, whose likelihood is influenced by prior consequences (6), points to its plasticity and forms the definition's center. The definition places no requirements on the form or physical structure of the response, its duration, the number of elements that can comprise it, or its complexity. It can be a sequence of units that takes hours to complete or a single discrete event such as a lever press.

This simple definition of operants has given rise to a powerful conceptual and empirical framework for studying and understanding behavior. Behavioral toxicology and the environmental health sciences have benefited from the basic understanding of operant behavior and the way in which it interacts with chemical exposure. The word *interaction* is important. The environmental condition supporting behavior and chemical exposure interact as equal partners in their influence over behavior.

Not all learned behavior is operant behavior. Other forms of learning include behavior changes that occur over the course of generations and are molded by natural selection as well as other behavior changes that occur within the span of a lifetime. The latter class includes changes in reflexes such as habituation, sensitization, reflex modification (7), as well as respondent conditioning (8). These simple forms of learning dominate the behavioral plasticity seen in invertebrates and assume a large role in the behavior of vertebrates. The emphasis here on operant behavior should not be interpreted as a diminution of the importance of other forms of behavior change.

The three-term contingency describing operant behavior identifies participants in the conditioning of operants: antecedent conditions, the operant itself, and the consequence. The relationship between the consequence and the occurrence of a response distinguishes operant from other behavior because it specifies the requirement that the response must occur for the consequence to act. This requirement is different from other forms of learning, such as respondent conditioning or reflexes, where the response is said to be drawn out or elicited by the stimulus. In the vernacular, operants constitute all voluntary responses, but without the teleology implicit in the word *voluntary*.

The elements describing operant behavior and the way in which they frame an understanding of chemical-behavior interactions are charted in Table 27.1. One element contains the condition's antecedent to a response. These antecedent condi-

TABLE 27.1. *Elements of operant behavior*

Element of the contingency	Element's role in ongoing behavior	Targets for chemical influence
Antecedent conditions	Stimuli contemporaneous with behavior Stimuli presented in the past Behavioral history Genetic history	Discrimination Sensory function Chemicals as stimuli Remembering Acquisition Species-specific effects
Operant	Physical characteristics	Nonspecific effects on response rate Motor effects of chemicals
Consequences	Reinforcing properties Aversive properties	Self-administration of chemicals Aversive properties of chemicals Chemical effects on punished responding
Combination of stimuli, responses, and consequences	Schedule-controlled behavior	Rate changes Disruption of response patterns Schedule-interactions with chemical class.

tions include the stimulus environment (including physical stimuli and temporal specifications) as well as behavioral, chemical, or genetic history. A second element is the operant itself, its physical makeup as specified directly or as it emerges, and the pattern by which it occurs. The consequence is the third element and identifies events produced by the response.

Chemicals can be a stimulus, a consequence, or a source of influence over all three elements. For example, the administration of a chemical can produce a private stimulus, such as lightheaded-ness resulting from inhaling solvents, that sets the stage for a particular response (9). A drug can also affect the manner in which stimuli control, or acquire control over, behavior and can affect discrimination, remembering, or learning. A chemical can be a consequence (10–12) or can influence the manner in which consequences affect behavior (13). Finally, a drug can have profound influences over the way in which a response is executed.

The three elements are always arranged in a schedule of reinforcement, which specifies how they interact. The operant and its rate and pattern of occurrence are extraordinarily sensitive to the specific makeup of the contingencies, or schedule, of reinforcement. Under time-based contingencies (e.g., a fixed interval [FI] 1 schedule, in which a reinforcer for the first response to occur after a minute has elapsed), characteristic patterns of responding occur in all normal, healthy mammals and birds studied, except sometimes in linguistically competent humans (14,15). If the contingency is response-based (e.g., a fixed ratio [FR] 20 schedule, in which a reinforcer is delivered after 20 responses, regardless of how long it takes), then a different type of responding is selected by the prevailing schedule. The specifics of the contingency, including the stimuli, the response, the consequences, and the temporal relationships among them all form the reinforcement schedule. The resulting behavior is called schedule-controlled operant behavior (SCOB).

The three elements can be individually perturbed by chemical exposure. In addition, the orderly behavioral patterns produced by schedules can be modified by chemical exposure and the specific disruption can depend on the prevailing reinforcement schedule. Because of these complexities, SCOB should not be reified as a single thing or a single process that is changed in a particular way by a toxicant or a drug. Doing so undermines the power of SCOB and trivializes the behavior under study. The schedule selects particular patterns of behavior, and a skilled practitioner can tailor behavior to meet the requirements of the question under investigation.

A large body of literature has evolved describing the pattern of responding selected by different schedules of reinforcement. A related body of literature has also evolved describing the interactions between the reinforcement contingencies and chemical exposure. The patterns of drug effects have been so consistent that chemicals can be distinguished according to their characteristic behavioral effects, and these distinctions correspond well to patterns seen at other levels of analysis, such as receptor binding assays (16,17).

Conditioning phenomena can contribute to an understanding of motor function because most motor acts are acquired or participate in behavior that is acquired. The emphasis seen in the behavioral pharmacology and toxicology literature, which apply conditioning principles to characterize chemical effects on behavior, has been on the interactions among stimulus conditions, consequences, and patterns of responding; but some attention has also been paid to the physical makeup of the operant (18). The domains of behavioral toxicology, motor function, and operant conditioning overlap. Each makes a unique contribution to our understanding of chemical effects on behavior, and each can be fully understood only in the context of the others.

DIMENSIONALITY AND THE MULTIPLICITY OF BEHAVIORAL MEASURES

The domain of motor events, like that of operants, can be described in the dimensions of time, displacement, mass, and different combinations of these, such as momentum, force, velocity, acceleration, and rate. Each dimension can, in principle, interact with other aspects of behavior, and the number of combinations can grow rapidly. Such proliferation of behavioral measures complicates analyses of motor function, especially when compared to the analysis of a single measure of behavior such as response rate.

Adding a new measure to an ongoing investigation may cost relatively little money, but it can complicate interpretations of effects that appear. If little is known about a chemical, then it might be necessary to incorporate several measures into an assessment even if doing so raises concerns about the interpretation of *statistically significant* effects that occur. Not least among the concerns is the statistical fact that when conducting multiple comparisons a number of statistically significant effects will appear randomly if adequate protection for overall error rate is not applied. A *P* value of 0.05 means that about 1 of 20 independent comparisons will, by chance, be judged to be statistically significant when in fact, they are not. If 100 comparisons are applied, then about 5 will be significantly different. A conservative approach to protecting for such chance effects, for example the Bonferroni tactic of dividing the *P* value by the number of comparisons, sacrifices power and raises the troubling possibility of missing a toxic effect, an error with potential consequences for the public health.

Slavish adherence to statistical considerations can result in a rigid decision to include only a limited set of measures and, regardless of the progress of the study, retain only those measures in all analyses. One could be put into the position, metaphorically speaking, of searching relentlessly for fleas, and ignoring the elephant strolling by because its appearance was not anticipated.

The solution to this problem is the topic of much debate in discussions about regulation (19,20,21), but such discussions are not new to science. To some extent these discussions reflect an increasing reliance on statistical inference to make our decisions for us (a reliance that is relatively new to science, see reference 22). Fortunately, other sources of judgment are available. Replication, for example, has a rich tradition and is far superior to statistical inferences for answering questions about robustness (23–25). Judgments about the plausibility of a pattern of effects are valuable. Effects that appear randomly due to multiple comparisons are unlikely to form a coherent pattern.

Even before these issues appear, information about how a chemical might act can reduce the number of dependent measures in a particular study. It is well known that knowing something about a chemical or the behavior under study can provide guidance in testing. Solvents can be expected to have one spectrum of effects, and basal ganglia insult is likely to produce something different. The structure of the chemical, the results of earlier tests, or reports from human exposure all can be incorporated into the design of an assessment strategy.

DIRECT AND INDIRECT EFFECTS OF REINFORCEMENT CONTINGENCIES

Reinforcement schedules have both direct and indirect effects on behavior. Awareness of the ways in which these schedules influence the execution of the operant can help when designing procedures for analyzing or when interpreting the motor effects of chemical exposure. Direct effects are formally specified in the definition of a schedule and can be used when synthesizing a response to be studied. For example, a direct effect of the FR schedule is that a certain number of responses precede the consequence. Indirect effects include functional relationships embedded in the schedule, without being an explicit part of the definition. They influence the rate, pattern, and topography of responding (26).

Direct Schedule Effects and the Synthesis of a Response

With repeated exposure to a reinforcement contingency, behavior conforms to the schedule requirement and forms an assay with which one can examine chemical effects. Direct effects of schedules can be exploited to synthesize a response that is defined precisely and whose perturbation can be interpreted. The particular assay depends on the chemical under investigation and the question asked. For example, it is relatively straightforward to reinforcing the pressing of a strain gauge within a specific force band to study sensory-motor function (18,27), to train an animal or a human to sustain a precise position so that tremor can be measured (28,29), or to track an object so that smoothness of movement and tracking abilities can be assessed (30,31).

If something is known about a chemical's likely effect on the physical structure of behavior then this knowledge could influence the design of the apparatus, the training protocol, and reinforcement contingencies. Together, these all contribute to the design of a response suitable for study. Careful planning could enhance the sensitivity of the protocol and facilitate one's ability to draw conclusions about mechanisms. Effects that may be subtle in animals but serious when humans describe them could be detected before widespread human exposure occurs.

Example: The Measurement of Tremor

The ideal test for assessing motor effects of chemical would be one that detects all neurotoxicants at the correct level of exposure without incorrectly identifying non-toxic chemicals. Such a test might be called *apical*, a term deriving from the same root as the apex, or top, of a mountain or pyramid. No such test exists, but a tremor appears with sufficient regularity to elevate its status toward that of an apical test.

Some change in tremor appears with striking frequency in neurologic disorders and with exposure to drugs and chemicals that act on the nervous system. Anger (1,2) has identified tremor as one of the most frequently cited perturbations associated with neurotoxicity, being associated with 177 chemicals or chemical groups. The only effects more frequently listed were weakness (179 entries) and convulsions (183 entries), but the amount of overlap among these categories is not stated. Given this, a protocol for detecting increases and decreases in tremor could be a useful addition to the armamentarium.

Tremor appears as regular oscillations in an efferent structure like a limb or the eye. In a neurologic examination, tremor might be detected by observing an outstretched hand or by asking a patient to perform a task like holding a full glass of water. Tremor can be characterized functionally according to what the limb is doing when tremoring is maximal: action, intention, postural, and resting tremor have all been identified and related to different types of nervous system damage (32).

When assessed in the neurologic screen, tremor is seen as a simple oscillation in the limb which can be described mathematically as one or several sine waves. When viewed quantitatively, tremor is rich phenomenon, and it is in such descriptions that its potential as an apical test may lie. Quantitative descriptions of tremor exploit spectral analytic techniques that transform a waveform that changes in time into a sum of sine waves of different frequencies (33).

Just as the conditions producing tremor can be diagnostic, so can the shape of the waveform describing tremor. With conventional screens, only simple increases in tremor can be detected reliably. Visible increases are large compared to normal tremor, however, and are often constrained to a narrow band of frequencies. For example, essential tremor and tremor enhanced by elevated levels of epinephrine (34) appear as large, easily detectable increases in the magnitude of 6-8 Hz oscillations, and the most visible aspect of parkinsonian tremor appears as large oscillations. More complex changes in tremor are difficult to detect by relying only on visible examination. Certain muscle disorders may appear in changes over a broad band of higher frequencies (35–37); and changes in tremor, such as those resulting from acute ethanol administration or myopathies, appear as *reductions* in tremor (36,28). Reductions in normal tremor may have important effects on such measures as reaction time, rapid muscle adjustments required to maintain posture, or movement speed, but reductions are difficult to see in a limb.

The multiple determinants of tremor may account for its frequent association with motor disorders. Freund et al. (36) points out that the bandwidth describing tremor (about 0 to 30 Hz) may contain frequencies of movement driven by different mechanisms. Slower frequencies probably reflect slow, dynamic adjustments in the total output of the motor neuron pool, whereas the higher frequencies could represent recruited, but unfused motor neurons. The frequency containing the most power, 6-8 Hz in normals about 3 Hz in Parkinson's disease for example, could represent the fastest speed that a limb can be moved voluntarily.

Tremor is not an operant, but its measurement requires the application of operant techniques to synthesize the appropriate response. With verbally competent humans, the application is achieved without even realizing that one is doing so. After all, people follow directions such as "Hold your arm horizontal and still." With animals and nonverbal humans, however, the measurement of tremor is facilitated by applying operant techniques to produce a baseline of limb steadiness so that small perturbations produced as tremor can be detected. Most measures of tremor, even electrophysiologic ones, can be facilitated by using reinforcement contingencies to synthesize the response of holding a limb steady so that tremor can be detected. The ability to combine measures of tremor with other measures of motor function and behavior in an intact animal could be a valuable component of an assessment strategy.

Adapting the response to the species can greatly shorten training time. In my experience, for example, training a squirrel monkey to execute a tracking task requiring precise movements takes a long time. Painful experience teaches that squirrel monkeys grab anything placed in front of their face, including fingers, so training one to grab a bar that it is facing is accomplished in a matter of minutes. Training squirrel monkeys to hold a bar relatively still for 8 seconds, which is enough time to evaluate tremor, is relatively straightforward and a suitable baseline can be established in a matter of weeks.

Figure 27.1 illustrates the control over bar-holding that can be established with a

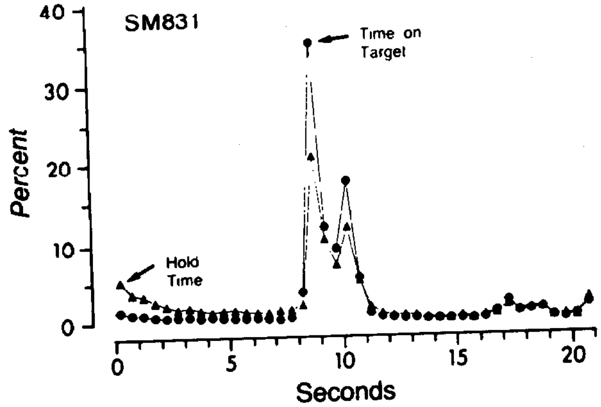

FIG. 27.1. Typical dwell time distributions illustrating the time spent during a session spent holding the bar (*circles*) and holding the bar on target (*triangles*) for a single monkey. The dwell time for a particular class interval was obtained by multiplying the number of episodes by the duration represented by class interval. From Newland and Weiss (28) with permission.

squirrel monkey trained to hold an L-shaped bar within a 4.5 cm band. The figure shows dwell times, or the percent of all time spent holding the bar that occupies a particular response class. About 35% of the total time that the bar was held within the 4.5 cm-wide target (labeled *time on target*) was spent in a sustained, bar-hold of 8.5 to 9 seconds and represents occasions when the monkey positioned the bar, held it until the reinforcement cycle began, and then let go. The mode at about 10 seconds represents times that the monkey held the bar through the reinforcement cycle. The small modes between 17.25 to 19.25 seconds represent occasions that the monkey held the bar for two successive reinforcers. Bar position was sampled for 5.12 seconds during criterion holds of at least 8 seconds, and the resulting signal was processed for later evaluation of tremor. The curve labeled *Holds* represents false starts or apparent slaps at the bar and was particularly sensitive to ethanol. The shape of the *time-on-target* curve was less affected by that drug.

The mathematics of converting position to acceleration are such that large, slow displacements can be tolerated without disturbing the measurement of tremor (33). A measure similar to that seen with an accelerometer can be derived from position, or displacement, D, as follows:

$$\begin{aligned} a &= \frac{d^2}{dt^2}\{D \sin(2\pi ft)\} \\ &= -D\,(2\pi f)^2 \sin(2\pi ft) \end{aligned} \qquad [1]$$

Using 9.810 mm/sec^2 as a milli-**g**, (9.81 m/sec^2 is 1 **g**, the acceleration due to gravity), the relationship between displacement, in millimeters, and acceleration is in equation 2:

$$\text{Displacement} = 9.81\,\frac{a}{(2\pi f)^2} \qquad [2]$$

Displacement is a direct function of acceleration at a particular frequency and an inverse function of the square of frequency at a particular acceleration. The latter relationship means that low-frequency displacements contribute little to changes in acceleration. Normal, physiologic finger tremor in humans is 10 to 30 milligrams at 7 Hz. The corresponding displacement is small, between about 0.05 and 0.2 mm, and is difficult to see without amplification. Narrow-band increases in tremor can be detected visually, although the magnitude and frequency bands of the increase might be difficult to determine precisely. Spectral analytic techniques are required to identify broad-band increases in frequencies, redistributions across the spectrum, or decreases.

Decreases from normal physiologic tremor would be difficult or impossible to detect without advanced techniques, but the effort may be worthwhile because decreases in tremor could reveal motor deficits of chronic illness or drug exposure (28,36). Persons with essential tremor are aware that very small doses of ethanol, such as a single glass of wine, can profoundly reduce tremor temporarily (39). Newland and Weiss demonstrated that even in normal squirrel monkeys, ethanol can also reduce tremor substantially in a dose-related manner (Fig 27.2). Tremor

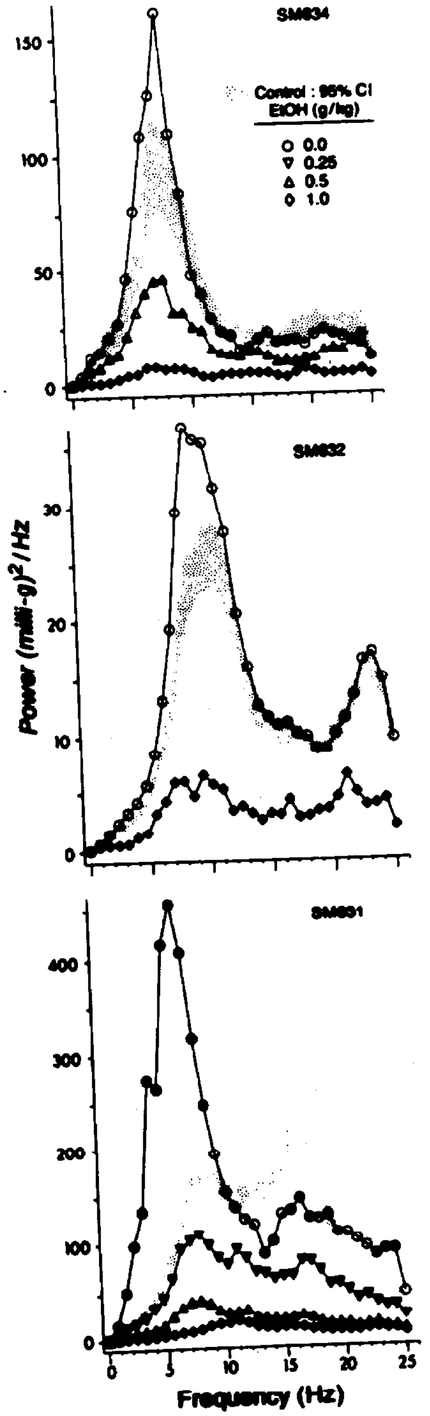

FIG. 27.2. Spectral analysis of the second derivative of bar position obtained after control, vehicle, and ethanol exposure. Confidence intervals of 95% (based on geometric means) are represented by the shaded areas. Symbols represent the geometric mean of the spectral estimate from vehicle sessions. Filled symbols are statistically distinct from control values. From Newland and Weiss (28) with permission.

may reflect normal functioning of stretch reflexes important in mediating posture, among other things. Tremor, and body sway tests, are both sensitive measures of ethanol exposure and represent some of the early motor effects of this drug.

Indirect Effects of Schedules

It is not always necessary to train explicitly a specific movement, as the physical manifestation of the operant is a consistent, indirect effect of many schedules of reinforcement. Indirect effects are sometimes so reliable that they even constitute part of the characterization of schedule performance (26). These indirect effects of schedules can be exploited to characterize the disruption of the execution of the operant by chemical exposure. Such a tactic could be handy if operant behavior is already part of a testing protocol. Simple measures, such as duration or interresponse times, might be reliable and valid end points. The extent to which such indirect measures are sensitive to motor deficits is not yet known, but there is much promise.

The precision (or variability) of a response emerges as a part of the behavior and appears to lie in the nature of the contingency. This applies to dimensions as diverse as interresponse times or even accuracy of match-to-sample performance (18, 39,40), pointing once again to the power and sensitivity of operant behavior. For example, response-based schedules select short and virtually invariant interresponse times, whereas interval-based schedules select moderate, highly variable interresponse times (40–42). A similar statement might also apply to response durations (18). Therefore, performance under these two schedules can be analyzed not just for overall measures of rate, but also for measures of the execution of response.

Figure 27.3 illustrates some indirect effects of the schedule of reinforcement as they appear in interresponse time (IRT) distributions taken from two schedules of reinforcement. Following tradition, the IRT was the time between the onset of a response and the onset of another response and, therefore, included response duration. (When response duration can be expected to vary in interesting ways, it should be separated from the IRT.) The IRT distribution from the FR component (top panel) contains a sharp, well-defined mode that appears at about 0.5 seconds. The small error bars, which indicate standard errors taken across five sessions, show little variability in the values of the bins and the location of the mode from session to session. The visible manifestation of this IRT distribution is a vigorous pattern of rapid responding: the monkey perched on the bar and repeatedly executed responses until the ratio requirement was fulfilled.

The FR 20 schedule directly specifies that 20 responses must occur before the reinforcer is delivered. The direct relationship between response rate and reinforcement rate acts at a molar level to elevate overall rate of responding. Perhaps more important, this schedule tends to deliver reinforcers in the middle of response bursts and thereby indirectly selects short IRTs (26,41,42). Under this schedule, high rates of responding that occur in bursts tend to appear.

The Fixed Interval (FI) 90 second schedule directly specifies that the reinforcer is

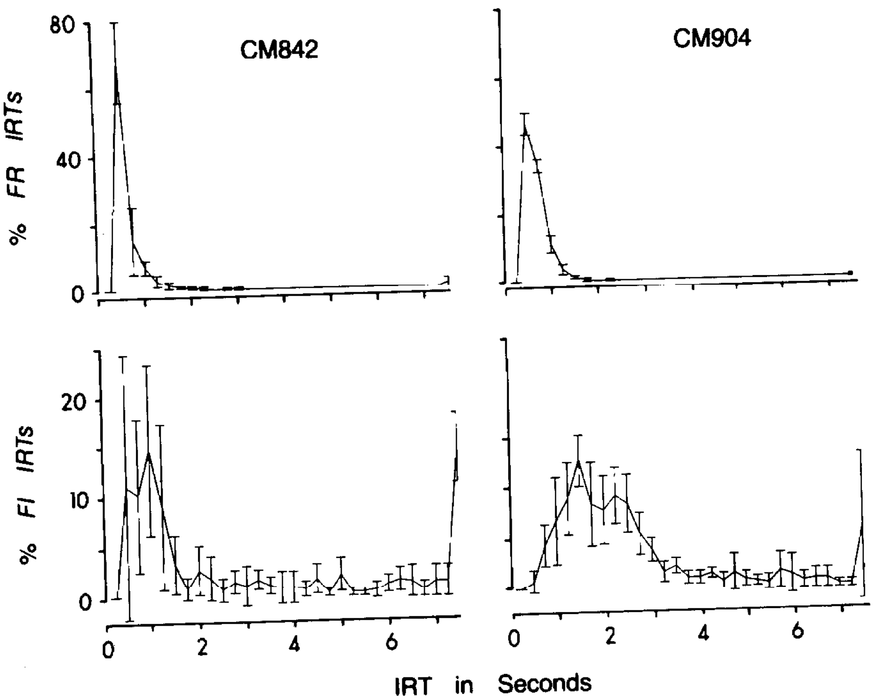

FIG. 27.3. Interresponse time (IRT) distributions in 0.25 second bins from five sequential, drug-free sessions for two cebus monkeys executing a rowing motion against a 40 Newton spring. Distributions from an FR20 schedule (*left*) and FI90 schedule (*right*) are shown. The distributions include both IRT and duration. Error bars show ± 2 standard errors of the mean. From Newland and Weiss (40) with permission.

delivered after the first response after 90 seconds elapsed. Typically, more responses occur than the one required and they assume temporal pattern of increasing response rates as the interval lapses. The weak linkage between the number of responses and the number of reinforcers delivered means that response rates can vary widely without changing the overall reinforcement rate. Perhaps this weak linkage is what makes FI-maintained behavior relatively sensitive to other influences, like the drug or toxicant exposure (16,43).

At the molecular level, interval schedules (e.g., FI, variable interval) can be viewed as more likely to deliver a reinforcer after a long IRT. The longer the IRT the more likely that the schedule has timed out and that the IRT will be followed by a reinforcer. The resulting IRT distribution is usually variable and dominated by moderate to long values (41). The distinction between the FI and the FR patterns of responding is visible in the IRT distributions, which were taken from the same monkeys and the same sessions. No clear mode appeared in the IRT distribution from the FI schedules. Instead, modes were smeared across a band of 1 to 2 or 3 seconds. A lack of session-to-session consistency appears as large error bars. Overlapping error bars indicate that the modal IRT from one session differed from that in a second session. The visible manifestation of this IRT distribution is of a more casual form of responding. The monkey sat in its chair and pulled on the bar intermittently. As the FI transpired, responses became more frequent but the vigorous pattern seen under the FR schedule never appeared.

There are many examples (16,43) in which drugs or toxicants change the rate of FI-maintained responding, usually by increasing rates, at doses that have little or no effect on FR-maintained behavior. With motor performance, however, the high rates and vigorous responding engendered by the ratio contingency may make the behavior maintained by ratio schedules sensitive to motor effects of chemicals.

Direct Effects, Indirect Effects, and Manganese Neurotoxicity

An investigation of manganese's neurotoxicity illustrates an approach to exploiting these different schedule effects (44). Manganese is a metal whose toxicity is largely neurobehavioral in nature. As with lead, there is relatively little concern about other organ systems or about carcinogenicity. Manganese's neurotoxicity appears, in advanced stages, as dystonic postures, action tremor, weakness, and, sometimes, psychiatric manifestations. Among the early signs and symptoms of manganese's neurotoxicity are complaints of fatigue, weakness, and clumsiness (45). Such effects have appeared in manganese miners inhaling high concentrations while working in dusty environments, but only after many years after exposure (47). This pattern of delayed toxicity presents a serious challenge to risk assessment strategy, especially one that is based on a single end point and a rigid statistical design. Some flexibility in the design of experiments might sometimes be necessary.

The experiments conducted to track the onset of manganese's toxicity in nonhuman primates were designed with the description of manganese miners in mind. The response device, designed by Jack Orr and Bernard Weiss, sustained executing a rowing-type movement through a displacement of 10 cm against a spring resisting movement with a force of 39 to 41 Newtons (3.9 to 4.1 kg, close to the animals' body weight) (illustrated in reference 40). The monkeys perched on a manipulandum and simultaneously pulled with their arms and pushed with their feet against a spring. Two response classes were specified. Completed responses moved through the 10-cm displacement and returned to the home position. Incomplete responses either did not traverse the entire displacement or did not return all the way to the home position.

Two schedules of reinforcement were used to maintain behavior: an FR and FI schedule. The FR schedule was applied because of the vigorous responding that it maintains. The FI schedule was used both because it maintains a different physical response and because response rate on this schedule is sensitive to chemical exposure. These schedules were imposed successively in the same session (technically, a multiple FR FI schedule).

Another consideration in designing the experiment was the temporal pattern of manganism: It is long-lasting, if not irreversible, and the signs tend to wax and wane. Such a pattern required the study of individual subjects over long periods of time. It was possible that a conventional design patterned after analysis of variance techniques would have missed some of these subtleties. Therefore, a multiple-baseline design, common to applications of behavior analysis was used (24,52). This design enables the comparison of an effect on an individual subject over a protracted time. Control conditions come from the subject's own baseline as well as that of other, untreated subjects performing the same task on the same day. The first comparison enhances sensitivity by reducing the contribution of intersubject variability in baseline levels of behavior. The latter comparison protects against influences that are irrelevant to the question at hand, such as changes in the apparatus, diet, season, or the light-dark cycle.

Figure 27.4 shows the effects of manganese on incomplete responses in monkeys executing a rowing-like motion against a 40 spring. Each point represents the number of incomplete responses taken from a single session, and the horizontal axis spans more than 400 days. The effect can be seen in a single monkey, CM904. Incomplete responses increased more than 100-fold after the first administration of 10 mg/kg of manganese. The other two monkeys were not administered the metal at this time, and their behavior did not change. The effect was replicated later in the other two monkeys, although it appeared less reliably in CM846. The incomplete response category measured as aspect of performance (one that might be called *motor precision*) has typically not been the focus of operant investigations. Interestingly, more traditional, molar measures of behavior, such as rate of completed responses, were not especially sensitive to manganese; however, another molecular measure, response duration, showed a pattern similar to that seen in incomplete responses.

Figure 27.5 illustrates median response duration during the FR-component before and after manganese exposure for the monkey least affected (CM846) by manganese on the number of incomplete responses. Duration changed subsequent to manganese administration, although the details of the pattern of change differed some from that seen with incomplete responses. In this case, duration was a more sensitive measure than incomplete responses (not the case for the other two monkeys in the study). Response durations may tap something similar to what the more direct measure of incomplete responses tapped; therefore, such a measure could be a useful complementary measure in studies in which motor function is not directly specified. The operant of moving the lever against the spring through a displacement was directly specified in the schedule, but the pattern of IRTs and durations that emerged was not.

A microanalysis of the duration and IRT of each response during the fixed ratio schedule was conducted on the monkey most affected by manganese (CM904) in the original paper (44). During baseline sessions, the 19 IRTs that separate 20

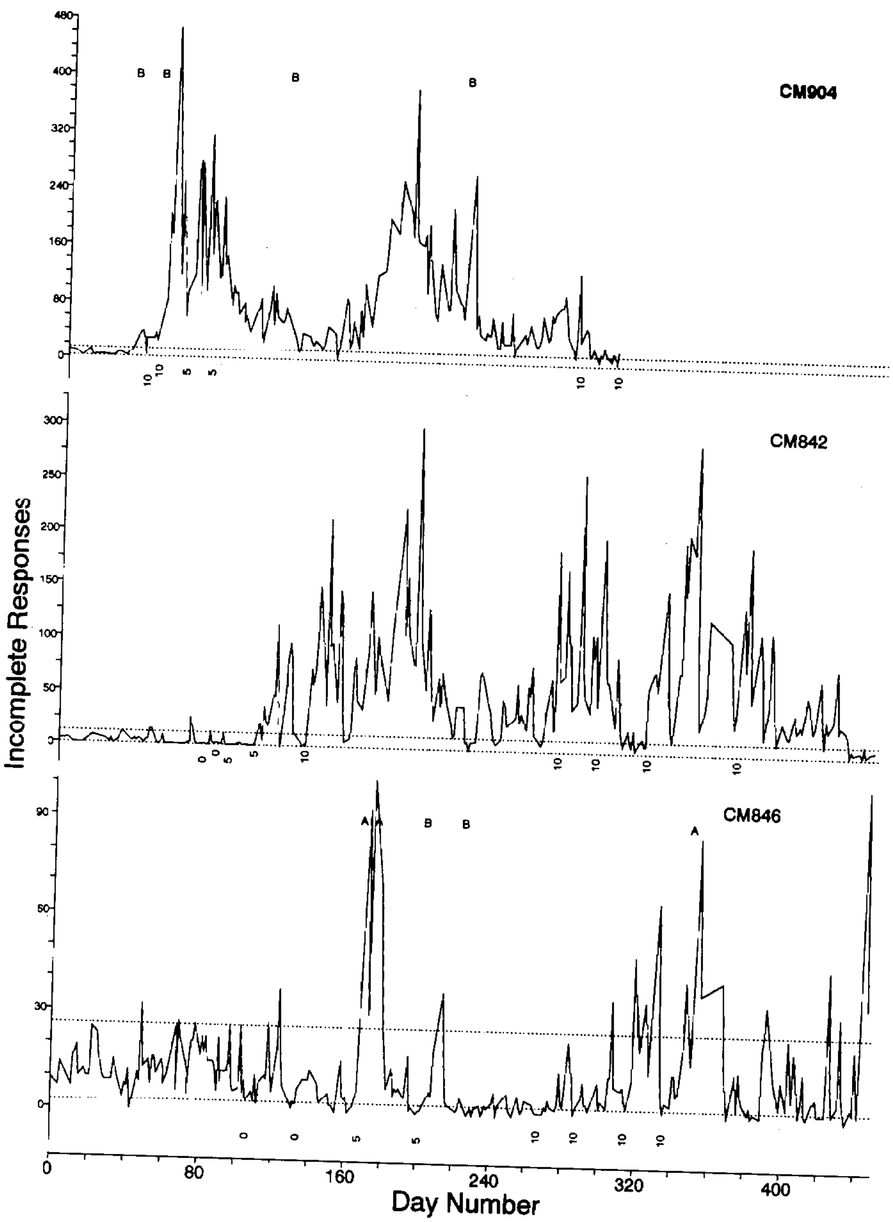

FIG. 27.4. Number of incomplete responses during the FR component for each monkey executing a rowing-type movement. The scales on the ordinates are different for each figure. Dotted lines show the 5th and 95th percentiles taken from baseline sessions. Day 0 for each abscissa is the same day. The number on the graphs shows the dose of manganese (as mg Mn/kg body weight, injected IV). Other letters indicate days when magnetic resonance images were taken of the monkey. From Newland and Weiss (44) with permission.

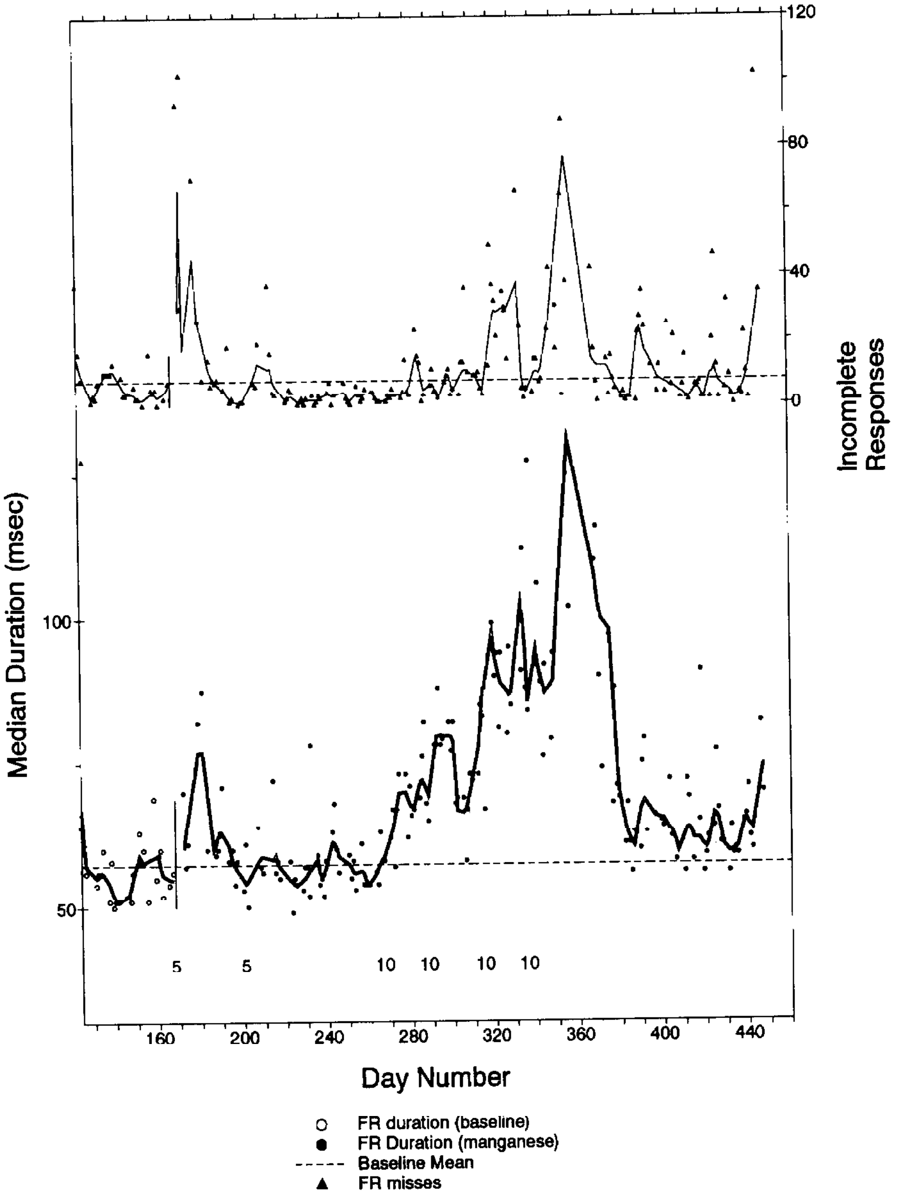

FIG. 27.5. Median response duration (*top curve*) and number of incomplete responses (*bottom*) for CM846, a monkey executing a rowing-type movement. The filled line is a smoothed, lowness, curve showing trends in the effect.

responses were about 0.1 second long, with little variation across different ratios in the session. A consistent pattern of durations also appeared: about 0.5 seconds for the first and last response and 0.3 seconds for the responses in the middle of the ratio burst. Manganese disrupted this pattern, and both durations and IRTs became erratic and highly variable. Ultimately, both measures assumed a new and different pattern, which may have revealed accommodation to impairment.

SENSITIVITY

Advanced techniques can be distinguished from screens, in part, on the basis of the degree to which direct manipulation is imposed. Advanced techniques impose greater experimental control, and one would expect greater precision to derive from such interventions. Such precision could entail detecting an effect at lower levels of exposure than seen on the screen, identifying effects with less variability, or failing to replicate an effect that appears on a screen. In the first case, improved sensitivity could afford better protection to the public by preventing subtle effects before they are experienced by people. In the last two cases, reducing variability and more precisely identifying effective doses can provide better, and perhaps higher, estimates of tolerable levels of exposure than those imposed by arbitrary margins of safety (Figs. 26.6 and 26.7).

Figure 27.6 compares several measures of motor function that have been related to ethanol exposure taken from selected studies. It sorts them according to the lowest blood alcohol level (where available or an estimate could be made) at which the effect appeared. In the study by Newland and Weiss (28) an estimate of blood alcohol levels was obtained by comparing the administered dose with the pharmacokinetics of oral ethanol in squirrel monkeys provided by Kaplan et al (46).

The range of blood alcohol levels at which effects become apparent is about 8:1 across the different end points. There is much intermingling of primate species across the different effects and rats were generally less sensitive. Screening tasks, which usually do not explicitly condition behavior, tended to be less sensitive. It is not clear how margins of safety will ultimately be applied in behavioral toxicity tests, but Fig. 27.6 suggests that concatenations of order-of-magnitude margins of safety, which might be applied to a new organic solvent incompletely tested, or assessed with rotor-rod measures, could ultimately be more expensive than actual testing of nonhuman primates at the low end of the concentration-effect curve.

Figure 27.7 compares two investigations of neurotoxicity in manganese. Because multiple measures of behavior were taken, it is possible to rank sensitivities of the different measures. One set comes from Suzuki et al. (60) who exposed eight monkeys (*Macaca mullata*) to manganese dioxide suspended in saline subcutaneously at different dosing rates. The end points were observations of neurologic signs. Another set comes from Newland and Weiss (44) who trained monkeys to execute an effortful response under FR and FI schedules of reinforcement as described earlier.

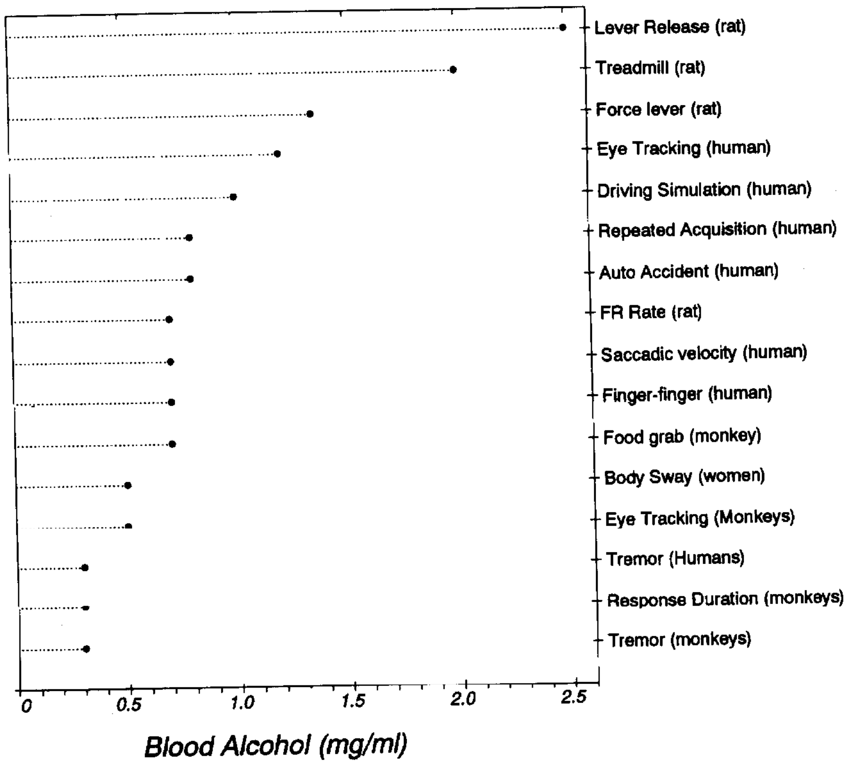

FIG. 27.6. Lowest blood level (measured or estimated) at which different ethanol-effects appeared in a variety of studies and species. [Lever release from (48), force lever from (27), human tremor from (50), eye tracking from (51), finger-finger task from (53), repeated acquisition from (54), eye saccades from (51), monkey tremor and response duration from (28), body sway from (38), FR rate from (56), food grab from (46), driving simulation from (58), auto accidents from (59)].

The latter experiment identified deficits in the execution of an effortful operant at doses 1/10 to 1/100 of the cumulative doses at which overt neurologic signs appeared in Suzuki's experiments. This is due, in part, to the different measures used: incompleted effortful responses and elevated durations versus observations of overt neurologic signs. Another influence, identifiable only because Suzuki et al. (60) carefully varied dosing rate, is the rate at which manganese is administered.

Some entries, such as IRTs and durations from the manganese experiments reported by Newland and Weiss (44) were not reported in that paper, but are presented here for comparison. An effect was said to occur if on at least 5 consecutive days, that response was outside of the 95% confidence intervals from baseline sessions. Incomplete responses and FR durations were elevated at low doses. Higher doses provoked action tremor and even higher doses produced dystonic postures as reported by Suzuki et al. (60) and Eriksson et al. (61). Some conventional measures, such as response rate, were virtually unaffected by manganese, and incomplete responses in the FI component were not affected consistently.

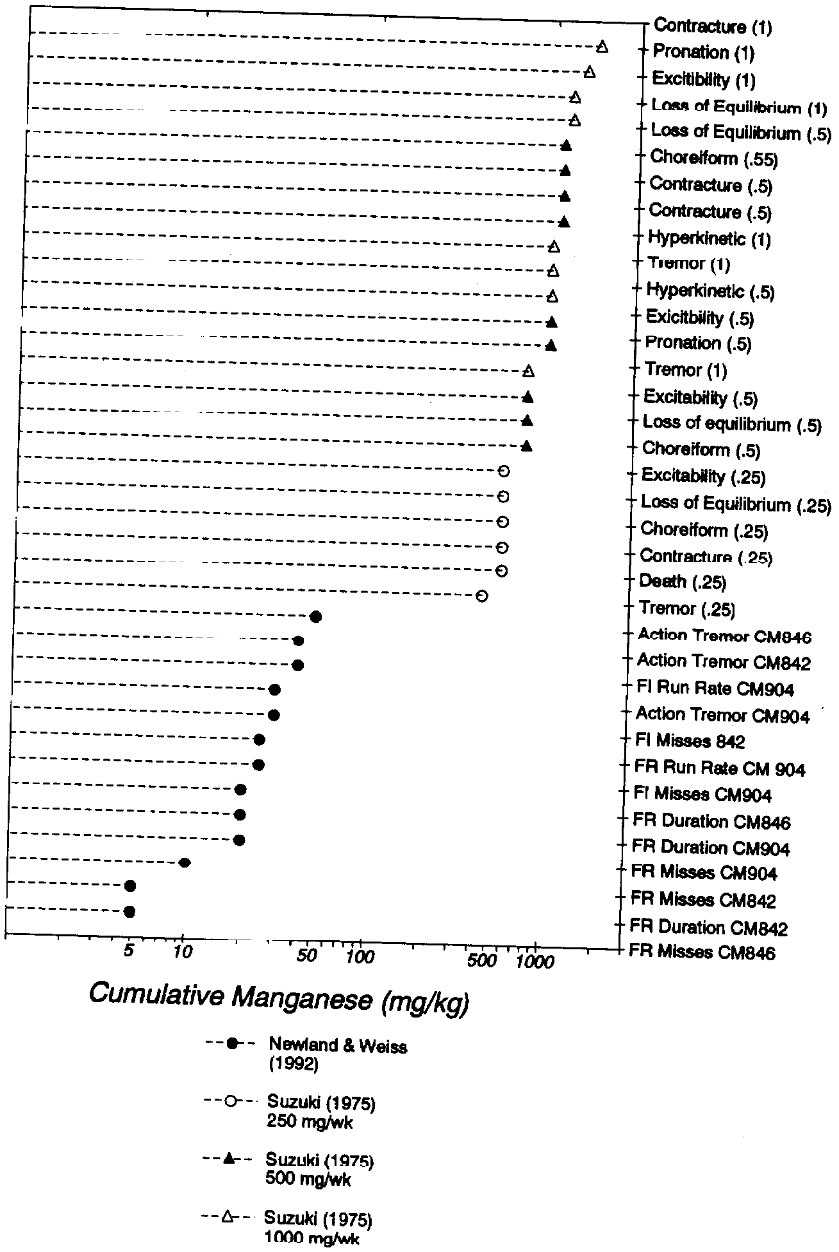

FIG. 27.7. Lowest cumulative manganese dose at which certain effects from Newland and Weiss (44) in filled circles and from Suzuki et al. (60), other symbols. Suzuki et al. (60) varied the dosing rate, and the different rates are indicated by different symbols. The dose scale is logarithmic.

The difference in the two studies is also related to the dosing rate, which can be seen in the dose at which tremor appeared. Briefly, the slower the dosing rate, the lower the dose at which signs appeared and the longer the delay to the appearance of overt neurologic signs. Newland and Weiss (44) observed tremor at doses of about 50 mg/kg, whereas Suzuki et al. (60) observed tremor at doses between 500 to 1000 mg/kg, depending on the size of the weekly dose. Other differences in the protocols of the studies, such as the form of manganese, route of administration, and species of monkey might have contributed to differences between their results.

These general effects have been replicated in other settings with human (45,46) and nonhuman (45,47,60,61) subjects. Eriksson et al. (61), using a higher dosing rate, reported these effects at twofold to fourfold higher levels of exposure than did Suzuki et al. (60). Newland and Weiss (unpublished data) reported them at lower cumulative doses, about 200 to 300 mg/kg subcutaneously using a lower dosing rate. The lower dose at which it appears may be related to the dosing regimen. The reason for the importance of dosing dynamics is not understood, but one possible mechanism seems reasonable. It may be that manganese, like other basal ganglia neurotoxicants such as methylphenyltetrahydropyridene (MPTP), destroys a small region of the basal ganglia and that signs do not appear until some threshold level of destruction is reached. In support of this contention is the observation that the latency to the effects in Suzuki's experiments was much longer at the lower dosing rates.

There is some doubt as to whether manganese's toxicity would ever appear as subtle changes in the rate or pattern of schedule-controlled behavior (44). Changes in rate or pattern alone, such as might be sought in some advanced tests, might miss manganese's neurotoxicity. If there is any clue that a chemical could affect motor function, then looking for it explicitly is indicated.

MECHANISMS AND REDUCTION

Questions of safety assessment are simplified when the effects of a chemical can be reduced to mechanisms of action, an endeavor in which otherwise disparate actions are described parsimoniously and causes are isolated. Marr (62), following Nagel (63), described two forms of reduction, heterogeneous and homogeneous, and this distinction is pertinent to toxicity assessment. Heterogeneous reduction includes the identification of neurochemical, pathologic, or neurophysiologic processes and draws explanations from "events taking place somewhere else, at some other level of observation, described in different terms and measured, if at all, in different dimensions" (64). Precise measures of behavior contribute to such reduction because the precision of the relationship that can be described between accumulation of a chemical in a structure and behavior change is related to the precision by which both behavior and the accumulation is measured. Clear specification of the behavior and the conditions under which it occurs is necessary in this endeavor.

Heterogeneous reduction requires the bringing together of behavioral technology and other approaches for studying neurotoxicity. Manganese neurotoxicity provides an example. The globus pallidus, substantia nigra, and pituitary gland probably mediate many of the early toxic effects of manganese. Manganese accumulation in the basal ganglia and pituitary gland can be tracked in individual monkeys using magnetic resonance imaging (MRI) (44,65). Because MRI is noninvasive, this technology enables repeated measures on an individual and, therefore, provides a powerful, sensitive, and efficient way to trace the onset and offset of the action of this metal *in individual subjects*. This revealed potential mechanisms for the neurotoxicity of this metal with the use of a very small number of subjects.

MRI technology was paired in these studies with a behavioral technology that was equally powerful, sensitive, and efficient (44,65). The application of behavioral techniques enabled repeated measures on individual monkeys, and conclusive effects were identified in a small sample (three subjects) of monkeys. The joint application of these two technologies, by performing MRIs on monkeys who completed the motor task on the day before and after the images were acquired, permitted direct links to be drawn between manganese's appearance in the globus pallidus and the appearance of motor effects.

Homogeneous reduction entails the identification of a general, parsimonious statement of events at the same level, using the same dimensions and terms used when describing the original events. Homogeneous reduction to mechanisms, like heterogeneous reduction, also provides a simple description of otherwise disparate actions. Examples of homogeneous reduction can be found in Newton's laws of motion or in the principle that the taking of drugs from many different pharmacologic classes is mediated by principles derived from the reinforcing properties of those drugs. Identification of behavioral mechanisms also exemplifies this form of reduction.

Behavioral mechanisms that might appear in the assessment of motor functions include behavioral compensation for damage and behavioral tolerance. These issues loom large in the assessment of motor function, as such compensatory adjustments could be responsible for the dynamic nature of effects; and manganese's neurotoxicity provides an example. Returning to Figs. 27.2 and 27.3, it can be seen that for both end points a temporal pattern appeared, spanning many weeks to several months. Effects grew smaller over the course of several weeks to months, but baseline levels were not necessarily reestablished. Microanalysis of the performance of one monkey (44) revealed that the pattern of response durations and interresponse times became severely disrupted by manganese but over several months settled down to a new, and different pattern. This pattern represented a behavioral accommodation to manganese's effects, and it appeared in an indirect effect of the schedule.

When investigating acute dose-effect relationships, behavioral tolerance or accommodation to impairment appears as a rightward shift in the dose-effect curve after behaving while influenced by the chemical. When investigating chronic exposure, accommodation might appear as a return toward baseline. In either case, such accommodation might entail acquisition of a new way of producing the response (44).

Screening and advanced strategies use both acute and chronic exposure regimens, each exhibiting the problem of behavioral accommodation. If the screening test uses behavior that the animal can perform frequently, then behavioral accommodation could develop in the cage, between tests, and an effect may not be detected. Behavioral tolerance is likely to appear when chemical-induced disturbance in function results in the loss of reinforcement, and accommodation may be similar. Behavioral tolerance has been reported in many aspects of chronic drug exposure and may be interpreted as a decline in the overall rate of responding as drug exposure begins and a subsequent increase if the rate reduction results in the loss of reinforcement.

CONCLUDING REMARKS

Many neurotoxic agents damage motor systems. The damage can occur at any of a number of possible levels and still appear in the execution of behavior. The assessment and understanding of disturbances in motor function can be aided by controlling behavior with operant techniques and applying concepts surrounding the explanation of behavior and behavioral change that has derived from the experimental analysis of behavior. Behavioral techniques can be used to synthesize a precise response directly. Responses can be differentiated to evaluate strength, motor control, or other physical dimensions of behavior. A precisely controlled response can also be useful for assessing neural processes, such as tremor, to isolate motor effects that could point to other mechanisms of damage. Another strategy might be to assess possible motor effects by incorporating them into other assessments that might already be in place. With the appropriate choice of behavioral baselines, different physical characteristics of responses can be selected through indirect or direct schedule effects. Whatever the strategy chosen, much of the behavior studied in protocols is conditioned behavior, even if the conditioning is not accomplished deliberately. Thus, ignoring schedule influences does not mean that they are absent, but only that the investigator has not accounted for them.

The initial expense of advanced assessments of motor function can be significant, although if incorporated into other protocols it may be minimized. Designing an apparatus, like those designed to sustain effortful responses or measure tremor, requires some setup and engineering costs, especially when commercial devices are not available. Nevertheless, such quantitative assessment of tremor is necessary to detect many effects or to reduce variability and uncertainty in the conclusions. Once the apparatus is constructed or tremor testing is in place, marginal costs may not be so great. The first set of data from such a preparation could be expensive, but subsequent ones would be more reasonable. Moreover, the expense incurred by an unnecessarily low threshold limit value could be large and widespread exposure to a toxicant that causes neurologic impairment can be significant.

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