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Comprehensive Observational Assessment: Ia. A Systematic, Quantitative Procedure for Assessing the Behavioral and Physiologic State of the Mouse*

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Summary. A systematic observational method is described for comprehensively assessing and quantifying the behavioral and physiologic state of the mouse and its response to drugs. With this method, the pattern profile of various classes of pharmacologic agents and their members can be identified and differentiated, and the relative specificity of their actions defined. The method is applicable to a wide range of investigative goals. Inter- and intra-observer reliability studies have shown it to meet the pragmatic requirements for research.

Key-Words: Mouse — Animal Behavior — Observational Measurement — Drugs — Biological Assay.

Because of prevailing views among pharmacologists that subjective measurement is necessarily unreliable, hence unscientific, observation rarely has been seriously approached as an instrument for quantitative measurement. Yet systematic observation offers the possibility for obtaining a broad range of information from a single system of measurement, for observing unanticipated treatment effects, and for obtaining information difficult or impossible to derive otherwise. Observation allows one to go beyond the limitations of a mechanical instrument. As noted by CLAUDE BERNARD (1865), observation is the "common ground of all our studies and explanations," providing the background information against which all experimental results and interpretations must eventually be reconciled.

Meaningful information and inference are rarely possible from a single unit of measurement; additional information is almost always required. The ideal methodologic approach for research is to obtain all

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such information simultaneously from the same subject, where each measure exists in quantitative relationship to the other and where the conditions for measurement are the same. Such quantitative relationship is impossible to achieve when the separate units of information are obtained from different subjects (IRWIN, 1962; IRWIN, 1968). The issue is particularly acute in pre-clinical drug evaluation where multiple information (as obtained through systematic observation) is required for human prediction. Investigators undertaking such study often complicate their problem (and reduce the value of their information) by measuring each behavior of interest separately, even intermingling different subjects, species, routes of administration and procedures of measurement in doing so. Under such circumstances, the investigator forfeits almost all possibility for making the kind of correlations and discriminations required.

The procedure that follows was developed to comprehensively assess the state of the mouse—behaviorally, neurologically and autonomically—to the extent possible through direct observation only. The object was to obtain sufficient information for recreating the animal's behavior from the data alone. It was first described at a Gordon Research Conference on Medicinal Chemistry at Colby Junior College in 1959 and has since been widely used—particularly by the pharmaceutical industry. Published descriptions of it, however, have been cursory or were made during an early stage of its development (IRWIN *et al.*, 1959; IRWIN, 1964). The present account is a detailed, complete revision of the procedure, broader in scope, more quantitative and more reliable. From the profiles of information obtained, as will be shown in subsequent publications, it has been found possible to distinguish between different strains of mice; most classes of psychoactive, neurologic and autonomic drugs; and the similarities and differences even between members of the same class of drugs. In addition, the procedure possesses low dose sensitivity so that "therapeutic-equivalent" drug effects can be discerned and quantitatively related to dose effects of an undesirable nature. It should be noted, however, that no claim to originality is made for most of the measures employed—only for their selection and integration into a single system of measurement.

The method is a complex one, requiring about two weeks to learn and an additional two weeks to develop assurance and ease in observation and animal handling[1]. Once learned, however, with suitable provision for "quality control," reliable, reproducible information can be obtained with it. A common rating scale is used throughout and approximately three minutes are required to process each animal. To allow for the

[1] A recommended procedure for training is available on request.

procedure's systematic use in standardized form, careful attention has been given to the details of scoring and processing of the animals. This paper presents the method, the time-dose-response effects of chlorpromazine, and the results of intra- and inter-observer and test-retest reliability studies (10 replications) with orally administered saline, and the hydrochlorides of meperidine, chlordiazepoxide and imipramine.

Methods

Physical Arrangement and Equipment

The apparatus includes three separate units positioned together: (1) a 3-sided, folding transparent lucite viewing arena (38 × 84 × 20 cm), with a horizontal wire secured across the right rear corner 15 cm above the arena floor; (2) a dark green rubberized linoleum floor mat within the arena, with a raised, circular wire-mesh grid (2.5 × 15.2 cm animal jar cover; 6.3 cm mesh) on the left side mounted over a disc of linoleum affixed to the mat, and (3) a platform to the rear of the arena with a removable stainless steel wire-mesh top (on which animals are viewed beneath 10 × 12.7 cm glass jars), a removable drop-pan for animal excrement underneath it, and an adjoining alley-runway (5 × 5 × 84 cm) just above and over the viewing arena.

The physical equipment required for testing were (1) a thin, *stainless steel plate* (11 × 15 cm) for transporting animals to the viewing arena without direct handling, (2) a #21 flexible *hypodermic needle stylet* (for eliciting pinna and corneal reflexes) attached at one end to a 5 cm length of wood dowel (6.3 mm diameter) sharpened to pencil-point at the other end for testing salivation and provoked biting, (3) a 15 cm *dissecting forcep* for eliciting toe- and tail-pinch responses, (4) a *lamp* for testing the light-pupil response, and (5) the *wire-mesh grid* mentioned above for testing grip strength.

Animal Scheduling and Processing

Female, Berkeley Swiss mice were ordered to arrive in the laboratory at least five days before intended use. The food and water of those selected for use (approximately 18 to 20 g body weight) were removed about 4 p.m. the preceding day and substituted with a 20 per cent glucose solution to minimize the stress and weight loss that would otherwise result from overnight fasting. The following morning the animals were systematically randomized and numbered (dye marking) to form three test groups of 6 animals each, one group for testing in the morning and the remaining two in the early and late afternoon. The 6 animals of each group were then randomly assigned treatments on a "blind" basis, e.g., placebo and five different doses of test drug. With

this arrangement the observer could assess three animals per day for each of six treatments, controlling time of day as a variable and any systematic or subjective bias in animal selection or testing.

The procedure required the 6 animals of each test group to be observed and manipulated at 30-min intervals until the time of peak drug action or longer, allowing 5 min for each animal assessment. To assure that they were similarly handled and equilibrated, the animals were sequentially removed from their group cage, assessed for their baseline behavior (omitting measures of body position, locomotor activity and respiratory rate), orally administered treatment and placed beneath their viewing jar at staggered intervals of 5 min each. By the time the sixth animal had been placed into position, the first animal was ready for its assessment 30 min post-drug, etc., until the time of peak drug action. After testing, the animals were numbered by ear punching and those of two adjacent treatment doses housed together (6 per cage) and observed over 7 days for toxicity and/or the duration of drug action.

Sequence for Observation and Manipulation

The procedure involved an initial phase of undisturbed observation and a later manipulative phase during which the animal was subjected to the least provoking stimuli first.

As shown in Figs. 1a and 1b, an animal's assessment always began by observing its undisturbed behavior within the viewing jar, i.e., *body position*,[2] *locomotor activity*, *bizarre behavior*, *exophthalmos*, *respiration*, *tremors*, *twitches* and/or *convulsions*. Thereafter, a metal plate was inserted under the glass jar and the animal transferred (as in Fig. 2a) and briskly dropped onto the floor of the viewing arena for testing *transfer arousal* and *spatial locomotion*. After this observation (10 sec duration), any *palpebral closure* present was noted (normally absent at this time)[3] and the *startle response* was elicited by snapping the fingers in front of the

[2] The measure *body position* with associated palpebral closure now supplants the former measure *sleep*, which was stringently defined as the animal lying in curled sleep-like posture with eyes closed. The new measures provide quantitative information on the general level of wakefulness.

[3] Animals normally exhibit increased respiration, exophthalmos and widened palpebral opening as part of the transfer-arousal response, making it a time particularly favourable for assessing palpebral closure and least favorable for other measures. The latter are best observed from the viewing jar where the animals are more prone to be inactive. Similarly, it is difficult to assess piloerection when the animals are in a crouched position, as often happens when in the viewing jar, in which case it should be scored during transfer arousal. As tremors are frequently exacerbated by handling or exertion, they also should be definitively scored during transfer arousal.

Fig. 1a and b. Summary of the sequence of behavior tested and the criteria for scoring

I. In Viewing Jar

Body position
0 Completely flattened
1 Lying on side
2 Lying upright
4 Sitting up
6 Standing on *HL* (rearing)
8 Repeated vertical leaping
P = Palpebral closure (0—8)

Locomotor Activity
0 None; resting
2 Casual scratch, groom/slow spatial
4 Vigor. scratch, groom/mod. spatial
6 Vigor. mvmt.; sl. sharp, rapid/dart
8 Ext. vigor. mvmt.; ext. sharp, rapid/dart
S Scratch; *R* Restless; *W* Writhe

Bizarre Behavior (0—8)

HF	Head Flick	*P*	Prancing
HS	Head Search	*UW*	Upright Walk
H	Hallucinatory	*AW*	Aimless Wander
B	Compulsive Bite	*C*	Circling
SB	Self-Destr. Bite	*W*	Waltzing
L	Compuls. Licking	*R*	Retropulsion
		D	Spat. Disorient.

Exophthalmos
+ Present

Resp. Rate/Arrhythmia

0	Arrest		
2	60/min	*L*	Labored
4	120/min	*R*	Retching
6	180/min	*D*	Dyspneic
8	240+/min	*G*	Gasping

Tremors
0 None
2 Sl. fine body tr. (1.5 mm)
4 Mod. coarse (3 mm) w. sl. impair. locom.
6 Mkd. coarse (4.5 mm) w. mod-mkd impair. locom.
8 Ext. coarse (6 mm) w. locom. impossible
E Exertion tr. *R* Rest tr.
H Head only *T* Tail only

Twitches
0—8 Sl., mod., mkd., ext.
(magnitude/freq.)

Convulsions

C	Clonic	*T*	Tonic
Cs	Clonic, Symmet.	*Tf*	Tonic Flexion
Rn	Running Excit.	*Op*	Opisthotonus
Ch	Champing	*Em*	Emprosthotonus
P	Popcorn	*Rr*	Rock & Roll
A	Asphyxeal	*Su*	Sitting Up
D	Terminal Death	*Pr*	Praying

II. In Arena

Transfer Arousal (appearance)
0 Coma/sl. vibrissae mvmt. only
1 Mkd. dulled; slow, sl. mvmt.
2 Mod. dulled; slow, mod. mvmt.
3 Sub-alert; sl. dec. mvmt.
4 Alert; active mvmt./slow freeze
5 Hyperalert; rap. mvmt./act. frze
6 Sl. excit.; sl. sharp/dart mvmt.
7 Mod. excit., sharp/dart mvmt. ("hypomanic")
8 Ext. excit., sharp/dart mvmt. ("manic")
C Catalepsy

Spatial Locomotion
Duration (0—4) times speed of movement factor:
Slow = 1
Active = 1.5
Rapid = 2

Palpebral Closure
0 Eyes wide open
2 1/4 closed
4 1/2 closed
6 3/4 closed
8 All closed

Piloerection
0—8 Sl., mod., mkd., ext.

Startle (jerk)
0 None
2 1/4 cm
4 1/2 cm
6 3/4 cm
8 1 cm or more

Alley Progression (cm)
D 4+ sec. delay; *E* Early exit
→ No exploration

Pelvic Elevation
0 Mkd. flattened
2 Barely touches
4 3 mm elev. (1/8″)
6 6 mm elev. (1/4″)
8 12 mm elev. (1/2″)
C Crouched; *H* Abnormal head position

Tail Elevation
0 Flattened
2 Horizontally extended
4 Diagonally elevated (45°)
6 Vertically upright (90°)
8 Diagonally retrograde (135°)

Finger-approach
0 None
2 Head mvmt. only; at distance
4 Mvmt. to finger; no contact
6 Contact; partially on finger
8 Completely on & explores

Finger-Withdrawal
0 None
2 Mkd. eye-squint only
4 Squint w. sl. head & body retract.
6 Squint w. mod. withdrawal
8 Continuous withdrawal
B Biting; *F* Freeze

Touch-Escape
0 None
2 Slow esc./sl. frze (firm stroke)
4 Mod. rapid esc./act. frze (light stroke)
6 Vigor., rap. esc. (light stroke)
8 Ext. vigor. run-escape (barely touch)

Ataxic Gait (lurch)
0 None
2 Slight, definite
4 Considerable; w/o fall
6 Marked; fall every 4—6 steps
8 Barely moves w/o fall

Hypotonic Gait
0 None
2 Sl. w. sub-norm. pelvis; *HL* sl. post.
4 Mod. w. low pelv.; *HL* sl. lat.
6 Mod. w. flat pelv.; *HL* mod. lat./post.
8 Ext. w. flat abdom.; *HL* ext. lat./post./drag

Impaired gait, other
St Steppage
Sp Spastic
W Waddling
Ds Dysmetric
Dk Duck-Walk
Sc Scissor

Limb rotation
0—8 Sl., mod., mkd., ext.
A Anterior
P Posterior
L Lateral
M Medial
F Forelimbs only

Total gait incapacity
0—8 Sl., mod., mkd., ext.

Positional passivity (no struggle)
2 Held by neck
4 Held supinely
6 Held by foreleg
8 Held by hindleg

Fig. 1b

III. Tail suspension

Visual placing (nose distance)
0 None, even after nose contact
1 After nose contact
2 After mkd. vibris. contact (6 mm)
4 After sl. vibris. contact; active (12 mm)
6 Before vibris. contact; active (18 mm)
8 Early vigor. extension, incl. *HL* (25 mm)

Grip strength (grid-grip resistance)
0 None
2 Sl. grip; semi-effective
4 Mod. grip; effective
6 Active grip; effective
8 Unusully effective

Body tone
0 Complete flaccid.; no return cavity normal
2 Sl. flaccid.; rapid or v. sl. slowed return
4 Sl. resistance
6 Mod. resistance
8 Ext. resistance; board-like

Hypothermia
+ Present

Pinna
0 None
2 Mod. retract./sl. brisk flick
4 Active retract./mod. brisk flick
6 Very brisk flick
8 Hyperact., repet. flick barely touch
W Body withdrawal response

Cornea
0 None
2 Sluggish closure
4 Active single eye-blink
6 Double eye-blink
8 Triple eye-blink

Toe-pinch
0 None
2 Sl. withdrawal
4 Mod. rapid withd.; not brisk
6 Brisk, rapid withdrawal
8 Very brisk w. repet. extens. & flex.

Positional struggle
0–8 Sl., mod., mkd., ext.
(Mean pinna-corneal & vis. placing)

Wire maneuver
0 Actively grasps w. *HL*
2 Mod. diff. grap w. *HL*
4 Unable grasp w. *HL*; sl. raises
6 Unable lift *HL*; falls 6–10 sec
8 Falls immediately
B Behavioral fall

IV. Supine restraint

Skin color
0 Ext. blanching
2 Mod. blanch; mod. pink tone
4 No blanch; sl.-mod. dusky rose
6 Deep dusky rose
8 Bright, deep red flush
C Cyanosis (0–8)

Diarrhea
+ Present

Limb tone
0–8 Sl., mod., mkd., ext. resist.

Abdominal tone
0 Completely flaccid; no return cavity normal
2 Sl. flaccid; rapid or v. sl. slowed return
4 Sl. resistance
6 Mod. resistance
8 Ext. resistance; board-like

Pupil size (dilatation)
0 Pin-point
.5 1/16th
1 1/8th
2 1/4
4 1/2
6 3/4
8 Fully
Op Opacity; *N* Nystagmus

Light-pupil
0 Absent
2 Mkd. sluggish
4 Mod. sluggish
6 Sl. sluggish
8 Very active

Lacrimation
+ Present
C Chromodacryorrhea

Salivation
0 None
2 Very sl. wet margin sub-maxillary area
4 Wet zone 1/4 sub-max. area
6 Wet zone 1/2 sub-max. area
8 Wet zone entire sub-max. area

Provoked biting
0 None
2 Slight; weak
4 Mod. active; not vigorous
6 Vigorous; not immediate or cont.
8 Ext. vigor., intense & continuous
(Mean dowel & tail-pinch)

V. In arena

Tail-pinch
0 No response
1 Very sl. mvmt., sl. freeze/vocal.
2 Sl. bite, escape/mod. frze/mkd. vocal.
4 Mod. bite, escape/abrupt active freeze
6 Vigorous biting/escape
8 Ext. vigor. biting/escape

Righting reflex
0 No impairment
1 On side 1–2 times
2 On side 3–4 times
3 On side 5 times
4 On back 1–2 times
5 On back 3–4 times
6 On back 5 times
7 Sluggish when placed on back
8 Absent when on back & tail-pinched

Throughout handling

Grasp irritability (Biting tendency)
0 None
1 Slight
2 Moderate
3 Marked
(Sum scores body tone, pos. passivity & supine restraint)

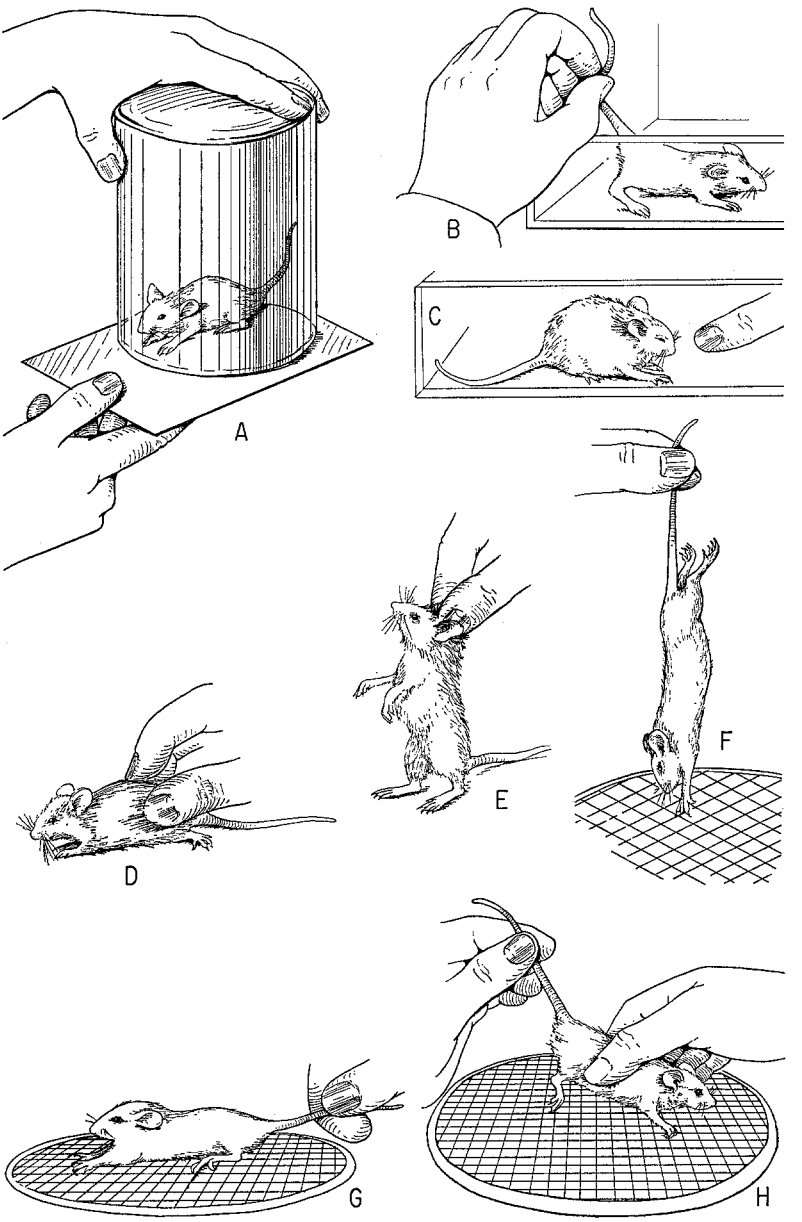
Provoked freezing
0 None
1 Slight
2 Moderate
3 Marked, abrupt freeze
(Sum scores transf. arousal, touch-esc. & tail-pinch)

Vocalization
f Number during handling

Urination-defecation
f Number during handling

Acute death
+ Present
C Convulsions; *R* Respir. depress.

Code *w.* with; *w/o* without; / or;
HL hindlimbs; *mvmt.* movement



a

Fig. 2a and b. Sequence of animal processing for the procedure

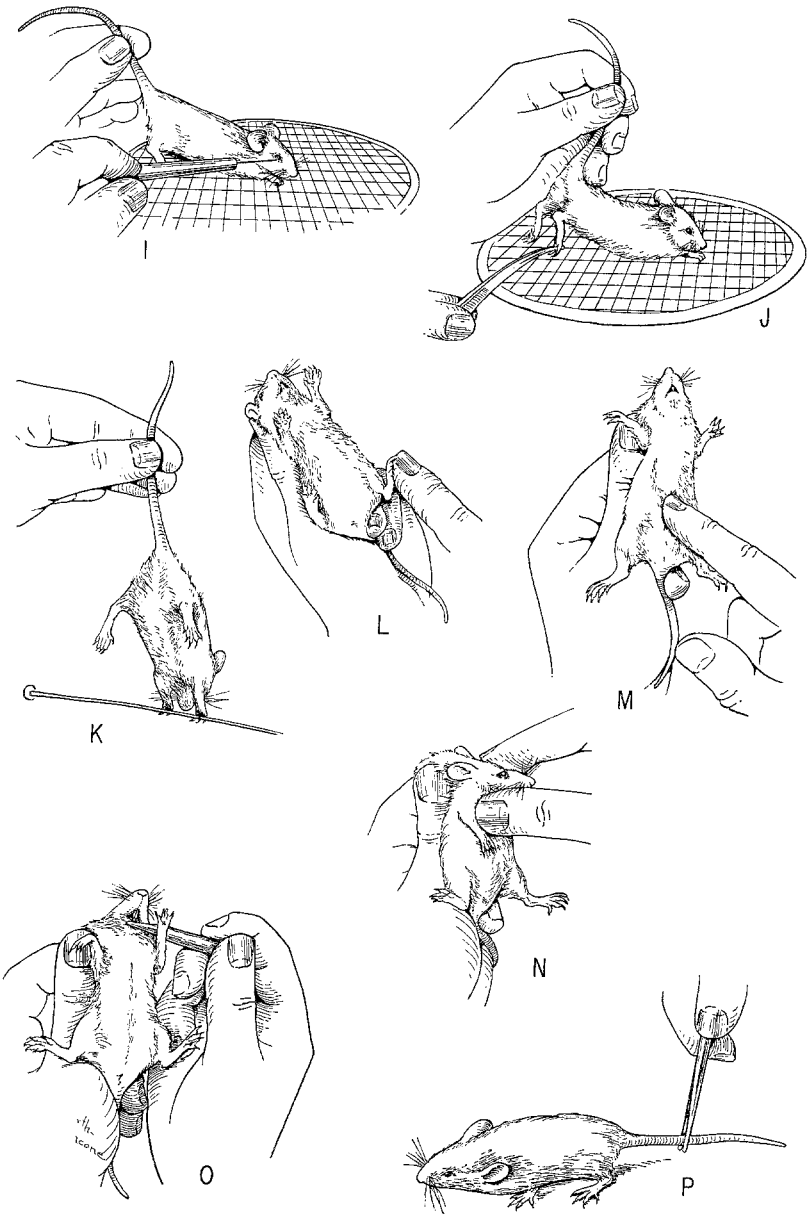


Fig. 2b

animal[4]. Observations were then made for *piloerection* and for more accurately discriminating the score for tremors when present. Throughout, if the animal was sufficiently mobile, observations also were made for *gait* and *limb rotation*; if not sufficiently mobile, these were made after touch-escape testing, at which time the animal could be provoked into movement by tapping its tail or compressing its sides.

The animal then was transfered by tail to the alley (Fig. 2b), and the distance transversed (*alley progression*) during the first 4 sec after initiating movement recorded; also noted were the *pelvic* and *tail elevation* during the course of movement. While still in the alley, but not in a corner, the animal was approached (approx. 1 sec) with extended index finger to within 3 mm for testing *finger-approach* or *withdrawal* responses (Fig. 2c). It was then transferred from the alley to the arena and stroked 3 times (from the lower thorax to the tail) by gentle pressure over the sides and back, for determining the *touch-escape* response (Fig. 2d). It was then brought forward, its movement arrested by pressure over the tail with the left hand, and a small fold of loose skin between and slightly below the ears grasped by the thumb and finger of the right hand for raising the animal vertically to test for *positional passivity* (Fig. 2e). If the animal excessively struggled or was too irritable to permit lifting, it was not tested further; the struggle response could be assumed to be present. In the absence of any struggle behavior, as shown in Fig. 4, the right hand was rotated so that the animal laid in supine position across the fingernails. In the further absence of struggle the animal was supported vertically by one forelimb, and thereafter by one hindlimb, to assess the degree of impairment present.

On completing the test for positional passivity, the animal was lifted vertically by mid-tail approximately 15 cm above the wire-mesh grid and lowered to elicit the *visual placing response* (Fig. 2f), usually characterized by an extension of both fore- and hindlimbs before contact. (The descent was decremental in speed, over approximately $1-{}^1/_2$ sec.) After contact with the grid, the hand was lowered to allow the animal to stand on all fours and a horizontal pull was applied to the tail to slowly draw the animal backwards (approx. $1-{}^1/_2$ sec) for a test of *grip strength*, i.e., the resistance of the animal to pull (Fig. 2g). This dual response test was then repeated, with observations made in addition for any *positional struggle* behavior while vertically suspended.

The animal was next held near the base of its tail, and positioned so that the forelimbs grasped the grid and the hindlimbs were slightly

[4] Observers unable to execute an adequate finger snap may substitute a light blow or puff of air over the face of the animal sufficient to produce an equivalent response.

elevated to barely touch it. Its *body tone* was then palpated three times by the thumb and index finger of the right hand through lateral compression between the lower thorax and pelvic region, the fingers not being removed between successive trials (Fig. 2h). Any gross *hypothermia* present was noted at this time. The *pinna* and *corneal responses* were then determined for the right, then left side by light tactile stimulation of the external auditory meatus and eyeball respectively with the hypodermic needle stylet (Fig. 2i).

Thereafter, while continuing to hold the tail, the hand was rotated so as to lift the tail and immobilize the spine through dorsal pressure with the remaining fingers. This positioned the hindlimb digits for the *toe-pinch response*, elicited by light compression of the lateral surface of the mid-digit of each foot with a forceps (Fig. 2j). During the pinna, corneal and toe-pinch testing, any *positional struggle behavior* was noted. (If the animal struggled excessively or became too irritable for such testing, the body tone, corneal and toe-pinch responses were assessed instead immediately after abdominal tone testing—at a time when the animal was physically restrained. The pinna response, however, could not be determined from this position and was omitted.) The animal was next brought to the horizontal wire, allowed to grasp it with its forelimbs, then released and observed for its *wire-maneuver* behavior (Fig. 2k). Thereafter, it was transferred to the grid for supine restraint.

When on the grid, the tail was held by the right hand with sufficient tension to allow the animal to be so firmly grasped by the loose skin of the nape with the left hand (just below the margin of the ears) that the head was immobilized. The tail was then transferred from the right to left hand, held firmly between the fourth and fifth fingers and the palm, and the animal rotated into a supine position of restraint. Observations were then made for *skin color*, *diarrhea*, and *limb tone*, the latter by pressing the tip of the index finger several times against the plantar surfaces of each hind foot (Fig. 2l). If the limbs were excessively flexed, the paw instead was grasped between the thumbs and index finger and alternately extended and flexed several times to determine its resistance to movement. *Abdominal tone* was then determined by similar palpation with the ball of the index finger (Fig. 2m), followed by observation of *pupil size* (Fig. 2n). If the pupil could not be observed because of eye-closure, the tip of the index finger was placed on the head of the animal and pressed downward while the thumb was brought into position just below the eye and the eye forced to protrude by moving the fingers slightly apart. If the pupils be markedly dilated, the *light-pupil response* was subsequently determined. *Lacrimation* and *salivation* were next noted, the mouth forced ajar by pressing downward against the lower jaw with the pencil-sharp end of the wood dowel for observing salivation

not visible externally (Fig. 2o). The dowel was then passed through the visual field of the animal and momentarily held in front of the mouth (approx. 1.5 mm) for the *provoked-biting response.*

The animal was then placed on the table top, briefly observed for its activity level, and moderate pressure applied with a forceps (Fig. 2p) about 2.5 cm above the base of the tail for determination of the *tail-pinch response* (4 sec), during which the animal was allowed to move about freely and additional observations made for any provoked biting or freezing. Thereafter, the animal was back-flipped 2 times (so as to somersault into the air two to three times each flip) approximately 30 cm above the table for determination of its *righting reflex*, the normal animal landing perfectly each time. If impairment was noted, it was flipped an additional 3 times for quantification. However, if the animal appeared markedly depressed or flaccid, its righting ability when placed on its back was noted first. If sluggish, even after firmly compressing its tail, the animal was not flipped.

During the entire process of handling, observations were also made for any *provoked-freezing* (during transfer arousal, touch-escape and tail-pinch testing), *grasp-irritability* (when testing for body tone, or grasped for positional passivity and supine restraint), *vocalization* and *urination-defecation.* On completion of these maneuvers, the animal was returned to its viewing jar, the observations recorded, and the entire process repeated for the next animal.

Measures and Criteria for Scoring

The measures and criteria for scoring are presented below. The symbol "#" designates measures in which only the occurrence of an event is recorded; the symbol "*f*", measures in which the frequency of occurrence per animal is recorded. An asterisk (*) indicates the desirability for using scores intermediate to those listed. A 0 to 8 range of scoring was used throughout, with values of 0, 2, 4, 6 and 8 representing none, slight, moderate, marked and extreme magnitudes of behavior. Figs. 1a and 1b summarize the rating scales employed. Where sufficiently described they are not replicated in the text that follows. The text is arranged logically to describe the behavioral, neurologic and autonomic measures separately, while the figures are arranged to describe the measures in their order of testing.

I. Behavioral

A. Spontaneous Activity

The undisturbed behavior of the animals in their viewing jars, exhibited just prior to testing for transfer-arousal.

1. Body Position[2]

Body position and palpebral closure, as described in the autonomic section, should be notated sequentially, e.g., 2P3.

*2. Locomotor Activity**

This is scored in terms of the speed and vigor of movement. The following activities, when present, should be identified by coded symbols and recorded, even if observed between testing:

a) *Exaggerated Scratching or Self Biting (S)*

b) *Restlessness (R)*, characterized by an appearance of uneasiness or discomfort, with inability to remain long in a given posture, e. g., pacing from side to side, repeatedly getting up and lying down or, if lying down, repeatedly shifting position.

c) *Writhing (W)*, characterized by an undulatory wave-like movement over the abdomen that involves a flattening or "sucking in" of the abdominal wall accompanied by asymmetrical stretching and extending of the body and hindlimbs.

3. Bizarre Behavior

The nature, occurrence and relative intensity of bizarre or stereotyped behavior on a 0—8 scale is recorded by the symbols designated below:

a) *Head Flicking (HF):* head shaking or backward flip of head.

b) *Head Searching (HS):* a stereotyped, repetitive turning of the head from side to side, as though searching the environment.

c) *Hallucinatory-Like (H):* behavior in which the animal appears to be responding to objects not present, e.g., visual tracking or fear-withdrawal.

d) *Compulsive Biting (B):* usually of the grid floor.

e) *Compulsive Licking (L):* usually of the glass jar.

f) *Self-Destructive Biting (SB):* usually biting of toes, with bleeding.

g) *Prancing Forelimbs (P):* restless shifting from one forelimb to the other, with slight turning of the body from side to side.

h) *Upright Walking (UW):* on hindlimbs only.

i) *Aimless Wandering (AW):* progressive, slow, plodding movements about the environment, with no apparent purpose.

j) *Circling (C):* tendency to move in circles around and along objects, or in an open environment.

k) *Waltzing (W):* rapid turning in circles.

l) *Retropulsion (R):* where the animal walks backwards.

m) *Spatial "Disorientation" (D):* walking or stumbling into objects.

Any other bizarre behavior noted should be described and its incidence recorded.

B. Motor-Affective Responses

These are evoked responses that reveal the emotional pattern of response of the animal to interaction with the investigator or environment, e.g., indifference, arousal, approach, avoidance, escape, vocalization or fighting behavior. For most of these, "normal" baseline scores differ so greatly among individuals and animal strains that they are not shown.

1. Alley Progression (Cm)

The animal is placed in the alley to the left of a starting point 10 cm from the end, and the distance in centimeters traversed (forward and/or backward) during the first 4 sec after the initiation of forward movement is recorded. Any delay (*D*) before movement exceeding 4 sec is noted, and animals failing to move within 10 sec are scored zero. The number of animals that traverse the alley *without exploration* (symbol →) or which exit (*E*) from the alley prematurely also should be noted, but the scores for the latter should not be included in the average.

*2. Transfer-Arousal**

This is scored in terms of the appearance of arousal (stupor, alertness or excitement) of the animal during and after transfer (10 sec) from its viewing jar to the arena, as shown below. The term arousal denotes the state of the animal along a coma-stupor-alertness-excitement continuum, "alertness" or "hyperalertness" representing the condition in which the animal is most attentive, vigilant and aware of its environment. Deviations from this optimum, i.e., toward stupor or excitement, result in diminished perception and awareness of the environment. With *stupor* (reduced arousal), the individual develops a relaxed, "dulled" facial expression, and appears less vigilant, aware of and responsive to environmental stimuli; enophthalmos is usually present. With *excitement* (increased arousal), the individual has a tense, "vigilant" facial expression, unusually sharp, rapid movements of the head or body and becomes increasingly less aware of the environment; exophthalmos with widened palpebral opening are usually present.

Stupor is usually associated with decreased activity and excitement, with increased activity. However, as following large doses of morphine, stupor can at times be accompanied by *increased* levels of locomotor activity; and excitement, as following large doses of amphetamine, by *decreased* levels of locomotor activity. Scoring, therefore, should *always* be based on the appearance of behavioral arousal of the animal, i.e., as stuporous, alerted or excited, rather than on the degree of locomotor activity present. Any unusual dissociation of locomotor activity and the state of behavioral arousal, however, should be noted and recorded.

Score	*Arousal Response to Transfer*
0	*Extreme Stupor or Coma:* Extremely "dulled," relaxed appearance; complete unresponsiveness during and after transfer, except for possible slight vibrissae movement.
1	*Marked Stupor:* Markedly "dulled", relaxed appearance; usually only very slight, slowed head or body movement.
2	*Moderate Stupor:* Moderately "dulled", relaxed appearance; usually slightly reduced vibrissae activity and moderately slowed and reduced head or body movement.
3	*Slight Stupor:* Sub-alert, relaxed, slightly "dulled" appearance; usually with active vibrissae and slightly reduced head or body movement.
4	*Alert:* Alert, calm appearance or slight freezing; exploratory head or body movements, when present, are active but not exaggerated.
5	*Hyperalert:* Hyperalert, vigilant appearance; active freezing or rapid (but not sudden or darting) head or body exploratory movements.
6	*Slight Excitement:* Slightly excited, tense appearance; with slightly sharp, sudden (or darting) head or body movements.
7	*Moderate Excitement:* Moderately excited, tense appearance; with moderately sudden, sharp, rapid (or darting) head or body movements; "hypomanic".
8	*Marked Excitement:* Extremely excited, tense appearance; with very sudden, markedly sharp, rapid (or darting) head or body movements; "manic".

Animals exhibiting 0 or 1 scores should be placed on a vertical wire grid. *Catalepsy (C)* is considered present if the animals retain their stance without falling or shifting location.

3. Spatial Locomotion

This is observed during the 10-sec interval for testing transfer arousal. It is scored on the basis of the total duration of progressive movement (walking or running) on a 0—4 scale multiplied by a factor for speed of movement as follows: Slow = 1, active = 1.5, rapid = 2, providing a maximum score of 8. The code letter "F" for fall is used to designate when animals run or walk off the table top without prior exploration.

*4. Touch-Escape**

This is tested by lightly stroking the sides and body of the animal three times from the region of the lower thorax to the tail. If stroked

from mid-thorax, the animal may tend to withdraw instead. The escape response (sometimes freezing) is scored in terms of its speed and intensity as follows:

Score	*Escape Response*
0	None; indifferent to the stimulus, so that no change in antecedent activity or locomotion is noted.
2	Slow or slight escape response or freezing when lightly stroked.
4	Moderately rapid escape or abrupt, complete freezing response when lightly stroked.
6	Vigorous, rapid escape response when lightly stroked.
8	Extremely vigorous running-escape response, even on approach or when barely touched.

5. Positional Struggle

This is the struggle behavior of the animals exhibited when partially or completely suspended by the tail during the pinna-corneal and visual placing tests respectively. The struggle behavior on each of the procedures is scored as slight (2), moderate (4), marked (6) or extreme (8), and subsequently averaged to obtain the final score.

6. Grasp-Irritability

This is the attempted nipping or biting behavior exhibited by the animals when tested for *body tone* or grasped and lifted by the scruff of the neck for *positional passivity* and *abdominal tone* testing. The score reflects the caution the investigator must exercise to avoid being bitten, and is scored as with provoked-freezing (below).

*7. Provoked Biting**

This score is derived from the averaged biting vigor of the animal in response to supine restraint and forced opening of the mouth with a wood dowel, and from the response to moderate forceps pressure applied approximately 2.5 cm above the base of its tail for 4 sec. (*Note:* The scale for biting in relation to tail-pinch scores should not be confused with the provoked biting scale.)

8. Provoked-Freezing

This represents the fear-arrest response of the animal, when present, during *transfer arousal*, *touch-escape* and *tail-pinch* testing. It is scored for each of the measures on a 0 to 3 scale. The individual scores are subsequently added to obtain the final score on a 0 to 8 scale, 8 being the maximum possible score.

*9. Finger-Withdrawal**

This represents the contact-avoidance or fear-withdrawal of the animal when the index finger of the investigator is slowly extended toward it (approximately 1 sec) and held for approximately 4 sec about 3 mm in front of the animal. If the animal retreats, the finger is moved along with it. If it should bite (*B*) or freeze (*F*), these should be noted.

*10. Finger-Approach**

This represents the exploratory approach of the animal when the index finger of the investigator is slowly extended toward it, as described for the finger-withdrawal response. It signifies contact-seeking and the relative absence of fearfulness.

*11. Positional Passivity**

This is scored on the basis of the struggle behavior (active, slight or none) exhibited when the animal is sequentially placed in positions in which the behavior is usually abolished by drugs: (A) suspended vertically by nape of the neck, (B) gentle rotation from vertical to horizontal position, so that the animal rests across the observer's nail-beds, (C) lifted and suspended vertically by one forelimb, and (D) inverted and suspended vertically by one hindlimb. To initiate the test the animal is gently restrained by pressure on its tail, then grasped by a fold of skin between the ears and slowly raised vertically. The response to position D is sometimes abolished before position C; in such instance, score D as though it were C. Otherwise, in testing, one should proceed *no further* than the position eliciting an active struggle response. Repeat the procedure to assure accuracy, taking the average response obtained as the score.

12. Vocalization (f)

The number of vocalizations exhibited by the animal during the process of handling is recorded. It usually signifies irritability and/or fearfulness.

13. Urination-Defecation (f)

The number of urinations and fecal boli exhibited during handling is noted and recorded as an index, in the main, of fearfulness.

C. Sensoro-Motor Responses

These are evoked responses which, in the main, reflect the level of perceptual awareness and responsiveness of the animals.

*1. Visual Placing**

The animal is lifted by the base of the tail to a height of approximately 15 cm and lowered to the wire-mesh grid within $1-{}^{1}/_{2}$ sec, deceler-

ating as the grid is approached. Scoring is based on the distance of the animal's nose from the grid before extending its forelimbs toward it.

*2. Tail-Pinch**

Response of the animal, after first establishing its pattern of movement, when moderate forceps pressure is applied about 2.5 cm above the base of the tail for about 4 sec. It is scored in terms of the vigor of the response, e.g., escape, biting, vocalization or freezing.

*3. Toe-Pinch**

A leg withdrawal response (ipsilateral flexor reflex) after lightly compressing the lateral surface of the mid-digit of each foot with a forceps. The score recorded is the overall response of both limbs.

*4. Corneal**

The blink or eye-closure response of each eye to light tactile stimulation of the cornea with a hypodermic needle stylet. The score recorded is the average for both eyes.

*5. Pinna**

The flicking or retraction of each ear in response to light tactile stimulation of the external auditory meatus with a hypodermic needle stylet (# 21). The score recorded is the averaged responses for both ears. If the animal exhibits body withdrawal (*W*) instead, this should be noted.

6. Startle

A sudden body jerking movement of the animal in response to a finger snap[4] (normal score approx. 1). It is increased by fearfulness or increasing CNS excitability, and its scoring is visually approximated in terms of the magnitude of the jerk response.

II. Neurologic

A. Posture

This reflects both the behavioral and neurologic state of the animal, since tail and pelvic elevation are usually increased by excitation or rigidity and decreased by stupor or flaccidity. It is evaluated, in the main, during forward movement of the animal.

*1. Pelvic Elevation**

The elevation of the abdomen from the alley during forward movement of the animal. It majorly reflects the limb position—its extension or flexion. The presence of a crouched posture (*C*) or abnormal head position (*H*) should also be noted.

*2. Tail Elevation**

This is scored during the forward movement of the animal; the scores tend to be lower when the animal is at rest.

*3. Limb Rotation**

Any abnormal rotation of the hindlimbs from a vertical stance is scored as slight (2), moderate (4), marked (6) or extreme (8), the more extreme scores involving total limb displacement or extension. The direction of rotation is recorded by code as anterior (*A*), lateral (*L*), medial (*M*) or posterior (*P*), and combinations of these used to better define the location. Should the forelimbs be affected to a greater degree than the hindlimbs, or otherwise require attention, this should be indicated by the code letter "F" preceding the score, e.g., F-2AL, signifying slight anterolateral rotation of the forelimb. The numerical scores are averaged in reporting out the data, regardless of actual position.

B. Muscle Tone

This reflects both the behavioral and neurologic state of the animal, increasing with apprehension or excitement and decreasing with relaxation. It is scored in terms of the relative presence of muscle resiliency (resistance to compression) or flaccidity (softness with continuing cavity deformation after compression).

*1. Body Tone**

This is determined by compressing the sides of the animal between the lower thorax and pelvis several times at approximately one second intervals, using the thumb and index finger. The fingers should not be removed between successive trials, as the animal usually tenses on the first trial and may do so again.

*2. Abdominal Tone**

The animal is restrained in supine position and the abdomen gently palpated with the index finger. The resistance to palpation is scored as for body tone.

*3. Limb Tone**

The animal is restrained in supine position and the tip of the index finger gently pushed against the plantar surface of each hindpaw several times to determine its resistance to passive flexion. If flexed or offering no resistance, the paw is grasped between the thumb and index finger and alternately extended and flexed several times. It is subjectively scored in terms of the maximum resistance to passive flexion or extension observed in either of the two limbs.

4. Grip Strength

The animal is allowed to stand on a wire-mesh grid and a horizontal pull applied to the tail to slowly draw the animal backwards (approx. $1-{}^{1}/_{2}$ sec). This is repeated and grip strength scored in terms of the grid-gripping resistance of the animal to pull.

*5. Wire Maneuver**

The animal is lifted by the tail, allowed to grasp the horizontal wire with its forelimbs, then rotated partially downward and released. The tendency of the normal animal is to actively grasp the wire with its hindlimbs as well. The behavior is scored in terms of the degree of impairment noted, with major emphasis on muscle weakness. If present, the animal should be retested for confirmation. If the animal appears to drop from the wire for reasons other than muscle weakness, e.g., behavioral (B), this should be clearly noted. The impairment is averaged for the animals, even where behavioral rather than neurologic deficit is involved.

C. Equilibrium and Gait

These are scored in terms of the degree of impairment or disability produced.

1. Righting Reflex

Impairment is scored in terms of the inability of the animal to land squarely on all fours when somersaulted into the air. The animal is held by the tail and back-flipped 2 times (to somersault 2 or 3 times each flip) approximately 30 cm above the table. If impairment is noted, it is flipped an additional 3 times and scored in terms of the number of times the animal lands imperfectly on its side or back. Should the animal exhibit considerable paralysis or incoordination, it should be placed on its back for initial testing; it is not subjected to flipping if sluggish or incomplete righting is observed. Since interest in this measure is with *neurological impairment*, the tail of the animals failing to respond when placed on their backs should be firmly compressed to be sure that the failure is a result of neurologic and not behavioral deficit (as with stupor).

*2. Ataxic Gait**

This results from an inability of the truncal, pelvic and limb muscles to move in unison, so that the animal tends to excessively sway, rock or lurch to the side as it proceeds forward and is variously unable to walk a straight line. It is not a result of reduced muscle tone (although often associated with it) and is best observed when the animal proceeds slowly away from the observer. To avoid judgmental error, the waddle-gait described below is included in this score. When necessary, the animal

should be encouraged to move by gently tapping its tail. The gait is scored in terms of the degree of lurching or staggering present.

*3. Hypotonic Gait**

This is an impairment of gait due to limb weakness or paralysis in which the animal is variously unable to support its weight but can proceed forward in a straight line without lurching. It may, however, be associated with ataxia, or the waddle-gait described below. It is scored in terms of the degree of limb weakness present.

*4. Impaired Gait, Other**

These are listed by code and only their occurrence noted. Any other types seen should be described.

a) *Steppage (St):* Due to paralysis of the muscles of dorsiflexion of the foot or toes, the animal drags its forelimbs in walking, walks on its knuckles, or lifts its forelimbs unusually high to avoid dragging its toes over the ground (spino-muscular involvement). Only this exaggerated form of the gait is recorded.

b) *Spastic (Sp):* Shuffling gait with legs rigidly extended and not lifted during movement. When severe, the animal may walk on tip-toe (cortico-spinal involvement).

c) *Waddling (W):* Lateral wobbling movements of the pelvis (posteriorly) somewhat resembling ataxia, but due to weakness of the gluteal muscles and never producing more than moderate impairment.

d) *Dysmetric (Ds):* Incoordinate movement with a coarse tremor due to overshooting goal and oscillating back and forth trying to reach it (cerebellar or posterior column involvement). The oscillatory tremor is best observed by placing the animal on the narrow elevated edge of the viewing arena, where effort is required for it to maintain its position.

e) *Duck-Walk (Dk):* An involvement of the hindlimbs in which the animal walks with adducted thighs, laterally extended legs and on tip-toe, causing it to assume a crouched posture (produced by narcotic analgesics).

f) *Scissor (Ss):* The forelimbs cross over in extension (in front of one another) due to marked spasticity and adductor hypertonicity, and the animal moves on the balls of its feet (cortico-spinal impairment).

5. Total Gait Incapacity

This is a subjective estimate of the total degree of incapacity present preventing the animals from motor performance. When present alone, the score for ataxia or hypotonic gait also reflects the total gait incapacity score. When combinations of gaits are present, however, the total gait incapacity score is likely to exceed the score of any single contributor.

D. CNS Excitation

*1. Tremors**

These are involuntary, purposeless, oscillatory movements which result from the alternate contraction of opposing muscle groups. They are differentiated into exertion tremors (*E*), observed only during movement, and tremors at rest (*R*), and are scored on the basis of their relative coarseness. If localized, e.g., to the head (*H*) or tail (*T*), the area of involvement should be indicated. Otherwise, overall body tremors are assumed.

*2. Twitches**

These are brief, coarse, involuntary muscle contractions which cause the animal to abruptly jerk or twitch its limbs and/or body. They are frequently a precursor to convulsions, and are subjectively scored on a 0 to 8 scale in terms of their magnitude and frequency of occurrence.

3. Convulsions (#)

These should be identified by code, as listed below, and their occurrence as well as any terminal death (*D*) noted. Once the animal develops a seizure, no other measurement short of the seizure itself, respiratory embarrassment and/or death should subsequently be made, unless it appears to reasonably recover.

a) Clonic-Type Convulsions

Convulsions with alternate contraction and relaxation of the voluntary muscles.

1. *Clonic (C):* A coordinated, unsymmetrical convulsion with natural, purposeful-like movements, e.g., "running", sometimes preceded by a running excitement *(Rn)*.

2. *Clonic, Symmetrical (Cs):* Repetitive symmetrical jerks or twitches of the limbs.

3. *Running Excitement (Rn):* Often accompanied by mild clonus or leading to a severe convulsion.

4. *Champing (Ch):* Clonus of the jaws only.

5. *Popcorn (P):* A seizure where the animal repeatedly "pops" into the air.

6. *Asphyxial (A):* A terminal clonic or clonic-tonic convulsion resulting from respiratory failure.

b) Tonic-Type Convulsions

Persistant contraction and spasm of a set of voluntary muscles.

1. *Tonic (T):* Typically a sustained extension of the hindlimbs, usually preceded by tonic flexion (*Tf*). The latter code is used if tonic flexion occurs without extension.

2. *Opisthotonus (Op):* A seizure in which the head, body and limbs are rigidly extended and arched backwards.

3. *Emprosthotonus (Em):* The opposite of opisthotonus, where the head, body and limbs are rigidly extended forward.

c) Miscellaneous-Type Convulsions

1. *Rock and Roll (Rr):* The animal is prostrate on its back and rocks from side to side in a seeming effort to right itself, occasionally rolling over (overshooting) and continuing to rock again.

2. *Sitting-Up (Su):* Where the animal sits upright on its hindlimbs during the seizure.

3. *Praying (Pr):* A sitting-up seizure in which the forelimbs are held together or crossed in an attitude resembling prayer.

III. Autonomic

A. Eyes

*1. Pupil Size**

The pupil size of the mouse in average lighting is about 1/8th dilated, i.e., about half the size of a pinhead. It is scored in terms of the total area of pupillary space occupied. Any eye opacity (*Op*) or nystagmus (*N*) noted should also be recorded.

2. Light-Pupil Response

A constriction of the pupil on sudden exposure to intense light. It is determined only when the pupil is markedly dilated.

*3. Palpebral Closure**

This represents a closure or drooping of the upper eyelids, usually associated with decreased sympathetic activity. It is determined when observing body position and again immediately after testing for transfer arousal.

4. Exophthalmos (#)

This is an abnormal protrusion or bulging out of the eyeball, usually associated with sympathetic stimulation. It should be observed when the animal is undisturbed, i.e., in the viewing jar; only its occurrence is noted. (Enophthalmos, a retraction of the eyeball, can be assumed present when ptosis predominates, just as ptosis is necessarily absent when exophthalmos is present.)

B. Secretion and Excretions

*1. Salivation**

2. Lacrimation (#)

Its occurrence only is recorded. The presence of chromodacryorrhea (*C*), red-colored tears, also should be noted.

3. Diarrhea (#)

This is indicated by a liquid stool; only its occurrence is noted.

C. Miscellaneous

1. Hypothermia (#)

A reduced body temperature, as determined by palpation; only its occurrence is noted.

2. Piloerection

Erection of the hair characterized by a ruffled fur and increasingly puff ball-like appearance, subjectively scored.

*3. Skin Color**

This is scored in terms of the color gradations seen about the plantar surface and digits of the forelimbs from extreme blanching (vasoconstriction) to extreme flushing (vasodilatation). The occurrence of cyanosis (*C*) also should be noted, i.e., a bluish discoloration, but as an independent measure on a 0 to 8 scale of intensity not to be averaged with the scores of animals not exhibiting cyanosis.

*4. Respiratory Rate/Arrhythmia**

This is scored with the animal in the viewing jar. Depending on the level of activity or rest, the normal respiratory rate of the mouse may vary between about 60 to 200/min. The character of the respiration also should be differentiated.

IV. Toxicity

A. Mortality

The antecedents of death, wherever possible, should be noted and recorded, e.g., convulsions (*C*) or respiratory depression with developing cyanosis *(R)*.

1. *Acute Death (#)*

Mortality that occurs within 24-hours post-treatment.

2. *Delayed Death (#)*

Mortality that occurs beyond the first 24-hours post-treatment.

Data Processing

The observations made for each animal are recorded on the form shown in Fig. 3, where the measures are arranged in the order in which they are quantified and space is provided for a description of any unusual drug effects noted. Provision on the data recording sheet is made for receiving information on all the treatments (maximum of six) and all of the animals receiving each treatment (three). The additional vertical lines are to facilitate data input by a key-punch operator for computer

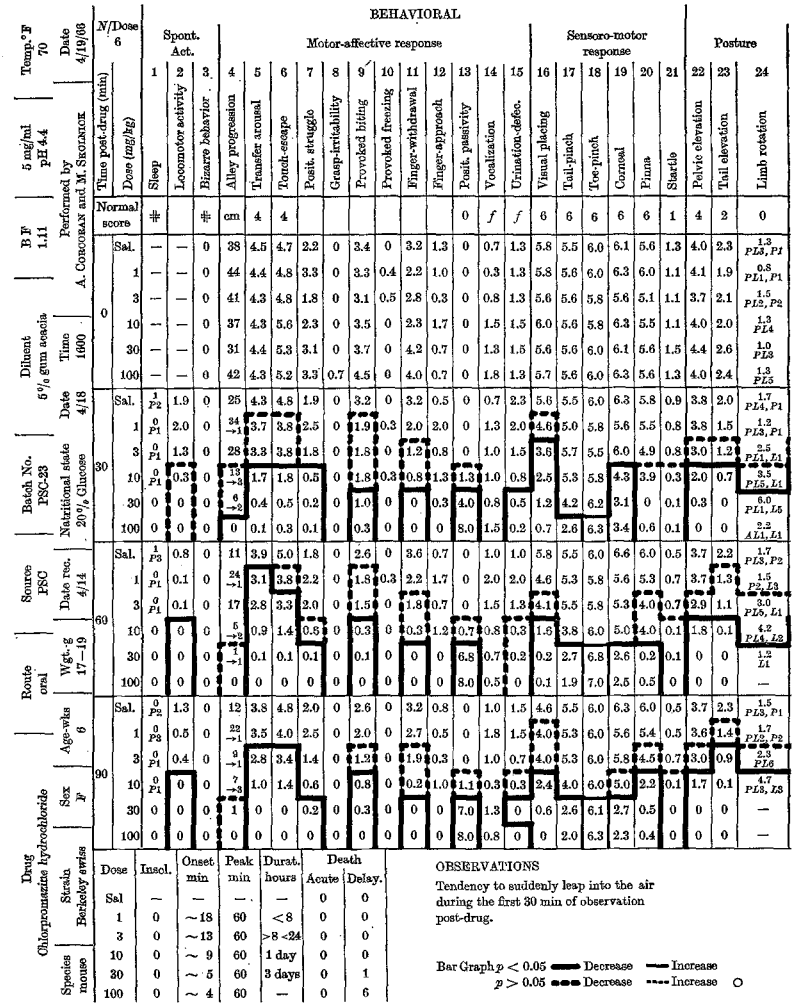
Post-drug: 60 min

Drug	Route	Source	Batch No.	Diluent		Mol. WT.
Chlorpromazine hydrochloride PSC-23	Oral	P. S. C.		5 % Gum acalia	5 mg/ml pH 4.2–4.6	$\frac{\text{Salt}}{\text{Base}} = \frac{355.3}{318.9} = 1.11$ B. F.

Species	Strain	Sex	Age	Date rec.	Nutritional state	Date	Time	Performed by	Temp. °F	Date
mouse	Berkeley Swiss	♀	5 wks	4.12.66	20 % Glucose	4.18.66	1630	M. S. Skolnick	70°	4.19.66

		Normal score				Mean				Mean				Mean				Mean				Mean				Mean
	Code/Color/Insol.		# 1/ 0				# 2/ 0				# 3/ 0				# 4/ 0				# 5/ 0				# 6/ 0			
	Dose-mg/kg		1				10				Saline				3				100				30			
	Animal No.		1	7	13		2	8	14		3	9	15		4	10	16		5	11	17		6	12	18	
	Body weight-g		15.8	18.6	15.3		16.4	16.3	15.6		17.4	17.0	16.2		17.2	18.8	16.6		17.5	18.7	15.4		16.2	16.4	16.6	
	Volume inj.-ml		.32	.37	.31		.33	.33	.31		.35	.34	.32		.34	.38	.33		.35	.37	.31		.32	.33	.33	
	Time administered		0950	1245	1440		0955	1250	1445		1000	1255	1450		1005	1300	1455		1010	1305	1500		1015	1310	1505	
Viewing jar	Sleep		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Palbebral closure	#	+	0	0	1	0	0	0	0	+	+	0	2	0	0	+	1	0	0	0	0	0	0	0	0
	Locomotor activity		0	0 5	0	0.2	0	0	0	0	0	0	3	1.0	0	0	0	0	0	0	0	0	0	0	0	0
	Bizarre behavior	#																								
	Exophthalmos	#																								
	Respiratory rate	4	4	4	3 5	3.8	2	2 5	2 L	2.2 L1	4	2 L	4 5	3.5 L1	4 5	4	2	3.5	2	2	2 5	2.2	3	2 5	3	3.8
	Tremors	0																								
	Twitches	0																								
	Convulsions	S																								
Arena	Transfer arousal	4	3 5	3	3 5	3.3	0 5	0 5	0 5	0.5	4	4 5	4	4.2	3	2 5	3 5	3.0	0	0	0	0	0	0	0 5	0.2
	Spatial locomotion																									
	Palbebral closure	0	0	1	1	0.7	4	3	5	4.0	0	0	0	0	0	2	3	1.7	6 5	5	6	5.8	6	5	5 5	5.5
	Piloerection	1	1	1 5	1 5	1.3	2 5	2	1	1.8	4	3	2	3.0	1	2	1	1.3	1 5	1	2	1.5	0	0	0	0
	Startle	1	1	0 5	0 5	0.7	0 5	0	0	0.2	0 5	0 5	1	0.7	1	0 5	1	0.8	0	0	0	0	0 5	0	0	0.2
	Alley progression	cm	2 9	2 4	1 0	2.1	1 6→	0	7→	8 →2	9	1 6	0	8	1 8	9	1 0	1.2	0	0	0	0	0	0	7→	2 →1
	Pelvic elevation	4	4	4	3 5	3.8	2 5	2 5	2 5	2.5	4	3 5	4	3.8	3 5	2 5	3	3.0	0	0	0	0	0	0	0	0
	Tail elevation	2	2	1 5	2	1.8	0	0	0	0	2 5	2	3 5	2.7	2 5	0 5	2	1.7	0	0	0	0	0	0	0	0
	Finger-approach		0	0	0	0	0	4	0	1.3	0	4	0	1.3	0	0	0	0	0	0	0	0	0	0	0	0
	Finger-withdrawal		4	4	5	4.3	0	0	2	0.7	5	0	5 5	3.5	4	2	4	3.3	0	0	0	0	0	0	0	0
	Touch-escape	4	4 ·	3	4 5	3.8	1 5	1	2 5	1.7	5	4	6	5.0	4 5	3	3 5	3.7	0	0	0	0	0	0	0 5	0.2
	Ataxic gait	0																								
	Hypotonic gait	0	0	0	0 5	0.2	6	5	6	5.7	0	0 5	0	0.2	0 5	5	1 5	2.3	8	8	8	8.0	8	8	8	8.0
	Gait, other	0	0	2 W	1 W	1.5 W2	2 W	2 W	3 4 W St	2.3W3 4St1	0	0	0	0	0	2 W	2 W	2 W 2	— —	— —	— —	—	— —	— —	5 5+	5.0 St1
	Limb rotation	0	1 L	2 L	2 P	1.7 L_2P_1	3 L	3 PL	2 L	2.7 L_2PL_1	2 P	2 PL	2 P	2.0 P_2PL_1	2 PL	4 PL	3 P	3.0 P_2PL_1	— —	— —	— —	—	— —	— —	7 L	7.0 L_1
	Total gait incapac.	0	0	0	0 5	0.3	6	5 5	7	6.2	0	0 5	0	0.2	0 5	5	2	2.5	8	8	8	8.0	8	8	7 5	7.8
	Positional passivity	0		0	0 0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	8	8	8.0	8	7 5	1	5.5
Tail suspension	Visual placing	6	6	3	3	4.0	2	3	2	2.3	5	7	6	6.0	6	4	3	4.3	0	0	0 5	0.2	0	0 5	1	0.5
	Grip strength	6	4	3	3	3.3	3	4	4	3.7	3	7	6	5.3	4	3	3	3.3	0 5	0 5	0 5	0.5	0	0 5	1	0.5
	Body tone	4	4	4	3 5	3.8	0 5	1 5	2 5	1.5	4 5	4	4 5	4.3	4	3	3 5	3.5	0	0	0	0	0	0	0	0
	Hypothermia	#				0	+	+	+	3				0				0	+	+	+	3	+	+	+	3
	Pinna	6	6	4	7	5.7	1	6	1	2.7	6	6	6	6.0	2	6	3	3.6	0	1	0 5	0.5	0	0	0	0
	Corneal	6	6	6	6	6.0	4	6	6	5.3	8	8	6	7.3	6	5	6	5.7	2	1 5	3	2.0	1	2	4	2.3
	Toe-pinch	6	5	6	6	5.7	6	6	6	6.0	6	6	6	6.0	5	6	6	5.7	7	7	7	7.0	7	7	7	7.0
	Positional struggle		1	1	2	1.3	0 5	0	0	0.2	1	1	2	1.3	3	2	0	1.7	0	0	0	0	0	0	0 5	0.2
	Wire maneuver	0	0	1	0	0.3	5	3	0	2.7	0	0	0	0	0	0	0	0	7	7	5	6.3	6	4	3	4.3
Supine restraint	Skin color	4	4	4	4	4.0	4 5	5	6	5.2	4	4	4	4.0	4	4	4	4.0	4 5	4	4 5	4.3	4 5	5	5 5	5.0
	Diarrhea	#																								
	Limb tone	4	3 5	2	3	2.8	0	2	1	1.0	2 5	4	4	3.5	4 5	3	4	3.8	0	0	0	0	0	0	0	0
	Abdominal tone	4	4 5	3	3 5	3.7	2	3	3	2.7	4	4	4 5	4.2	4	3	3 5	3.5	0	0	0	0	0	0	0 5	0.2
	Pupil size	1	2	1	1	1.3	1 5	1	1 5	1.3	1	1 5	2	1.5	1 5	1	1 5	1.3	1	1 5	1	1.2	1	0	1	0.7
	Light-pupil	8																								
	Lacrimation	#																								
	Salivation	0																								
	Provoked biting																									
Arena	Tail-pinch	6	5	5	6	5.3	2	2	6	3.3	7	7	7	7.0	6	6	6	6.0	1	1 5	0 5	1.0	2	4	2	2.6
	Righting reflex	0	0	0	0	0	3	2	2	2.3	0	0	0	0	0	0	0	0	8	8	8	8.0	8	2	3	4.3
	Grasp-irritability	0																								
	Vocalization	f	8	0	4	4.0	0	0	2	0.7	0	0	6	2.0	6	0	3	3.0	0	0	0	0	2	1	1	1.3
	Urination-defec.	f	3	2	1	2.0	0	0	0	0	1	1	0	0.7	2	2	3	2.3	0	0	0	0	0	1	0	0.3
	Provoked freezing		2	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Misc.	Death	#																								
Comment	+ = Present; — = Unable to measure; Blank Space = Normal Score																									

Fig. 3. General activity and acute toxicity. Protocol sheet for recording the scored data: The thin vertical lines are to facilitate data input by a key-punch operator for computer processing and denote the decimal point position. Measures exhibiting no deviation from "normal scores" are left blank



Dose	Insol.	Onset min	Peak min	Durat. hours	Death Acute	Death Delay.
Sal	—	—	—	—	0	0
1	0	~18	60	<8	0	0
3	0	~13	60	>8 <24	0	0
10	0	~ 9	60	1 day	0	0
30	0	~ 5	60	3 days	0	1
100	0	~ 4	60	—	0	6

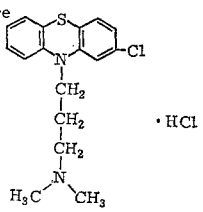
Fig. 4. General activity and acute toxicity. Time-Dose-Response Summary Form showing the averaged data scores

processing, and to denote the decimal point. Animals 1 to 6 represent the first group, 7 to 12 the second group and 13 to 18 the third group observed, one being tested in the morning and the remaining groups in the afternoon. A separate sheet is used to record the results of each interval of measurement, e.g., at 0-, 30-, 60- and 90-min post-drug, and provision is made for averaging the results of each treatment. To reduce the time involved for recording the data, only scores deviating from "normal" are recorded; measures exhibiting no significant change are left blank.

NEUROLOGIC													AUTONOMIC										MISC.				
Muscle tone					Equilibrium and gait					CNS excit.			Eyes				Secret. a excret.			General				Tox.				
25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Body tone	Abdominal tone	Limb tone	Grip strength	Wire maneuver	Righting reflex	Ataxic gait	Hypotonic gait	Gait, other	Total incapacity	Tremors	Twitches	Convulsions	Pupil size	Light-Pupil	Palpebral closure	Exophthalmos	Salivation	Lacrimation	Diarrhea	Hypothermia	Piloerection	Skin color	Respiratory rate	Acute death				
4	4	4	6	0	0	0	0	0	0	0	0	#	1	4	0	#	0	#	#	#	1	4	4	#				
4.6	4.6	3.8	5.6	0	0	0	0.5	0	0.5	0	0	0	1.7	4.0	0	0	0	0	0	0	—	3.8	—	0				
4.9	5.0	3.4	4.3	0.2	0	0	0.3	0	0.3	0	0	0	1.3	4.0	0	0	0	0	0	0	—	4.0	—	0				
4.7	4.8	4.2	5.2	0	0	0	0.4	2.9 *DK1*	0.4	0	0	0	1.6	4.0	0	0	0	0	0	0	—	4.0	—	0				
5.0	4.8	3.9	5.3	0	0	0	0.3	0	0.3	0	0	0	1.1	4.0	0	0	0	0	1	0	—	3.7	—	0				
4.8	4.8	3.8	4.8	0	0	0	0	0	0	0	0	0	2.1	4.0	0	0	0	0	0	0	—	4.2	—	0				
4.9	4.8	3.7	5.1	0	0	0	0.1	0	0.1	0	0	0	1.7	4.0	0	0	0	0	0	0	—	4.0	—	0				
4.5	4.5	3.7	5.5	0	0	0	0.5	0	0.5	0	0	0	1.7	4.0	0	0	0	0	0	0	2.2	4.2	4.1	0				
4.5	4.7	3.4	3.8	0.2	0	0	0.3	2.0 *W1*	0.3	0	0	0	1.4	4.0	0.9	0	0	0	0	0	1.0	4.0	4.3	0				
3.7	4.2	3.8	5.0	0	0	0	2.3	2.2, *W6* 2.0, *DK1*	2.3	0	0	0	1.3	4.0	1.3	0	0	0	0	0	1.1	4.0	3.9	0				
2.7	3.0	1.3	3.5	1.3	1.4	0	4.5	2.8 *W4*	4.9	0	0	0	0.8	4.0	3.5	0	0	0	0	3	1.4	5.3	4.3	0				
0.8	0.9	0.1	1.5	5.3	4.2	0	7.8	5.8 *St3*	7.5	0	0.3	0	1.4	4.0	5.7	0	0	0	0	5	1.8	5.0	4.4	0				
0.4	0.2	0	1.1	6.8	4.3	0	8.0	—	8.0	0	1.8	0	2.1	4.0	5.4	0	0	0	0	6	0.8	5.2	3.4	0				
4.3	4.3	3.6	5.6	0	0	0	0.8	0	0.8	0	0	0	1.8	4.0	0	0	0	0	0	0	2.8	5.0	3.8	0				
4.3	4.2	3.8	3.6	0.2	0.2	0	0.6	0.8 *W3*	0.7	0	0	0	1.4	4.0	1.2	0	0	0	0	0	1.5	4.0	3.8	0				
3.6	3.9	3.3	4.3	0	0.5	0	2.5	2.4, *W5* 2.0, *DK1*	2.6	0	0	0	1.4	4.0	1.7	0	0	0	0	0	1.0	4.0	3.9	0				
1.5	2.3	0.9	3.2	2.8	2.4	0	6.0	2.5, *W4* 1.0, *St1*	6.3	0	0	0	1.0	4.0	4.5	0	0	0	0	6	1.3	5.2	3.8	0				
0	0.1	0	0.4	5.5	5.2	0	8.0	5.0 *St1*	7.9	0	0.3	0	2.3	4.0	5.8	0	0	0	0	6	0.8	5.0	3.9	0				
0.1	0	0	0.4	6.7	7.3	0	8.0	—	8.0	0	0	0	1.8	4.0	6.1	0	0	0	0	6	0.8	4.3	3.0	0				
4.0	4.1	3.8	5.1	0	0	0	0.7	0	0.8	0	0	0	1.9	4.0	0	0	0	0	0	0	1.9	4.7	4.0	0				
3.8	3.9	3.3	4.0	0	0.2	0	0.8	1.5 *W2*	0.8	0	0	0	1.7	4.0	0.7	0	0	0	0	0	1.5	4.0	3.8	0				
3.3	3.8	2.8	4.2	0	0.4	0	2.3	2.2, *W5* 2.0, *DK1*	2.3	0	0	0	1.7	4.0	1.4	0	0	0	0	0	1.3	4.0	3.1	0				
1.6	2.1	0.5	3.6	3.2	1.3	0	5.9	2.2, *W4* 2.0, *St2*	6.2	0	0	0	1.3	4.0	3.9	0	0	0	0	3	0.5	6.2	3.9	0				
0	0.2	0.1	0.6	4.8	4.8	0	8.0	—	8.0	0	0	0	2.1	4.0	5.8	0	0	0	0	5	0.5	4.8	3.5	0				
0.1	0	0	0.4	6.9	7.2	0	8.0	—	8.0	0	0	0	2.0	4.0	6.4	0	0	0	0	6	0.8	4.3	3.1	0				

ASSESSMENT Major tranquilizer (chlorpromazine-type) with peak effects at 60 min and 8—24 hour duration of action depending on dosage. Slight effects with muscle tone reduction at 1—3 mpk; marked depression with mkd. ptosis, hypothermia, muscle tone reduction and hypotonic gait at 30 mpk; prostration with delayed mortality at 100 mpk.

Structure



A dash line (—), however, is used to designate information that could not be obtained, as with the pinna response with an overly active subject.

After completion of the study, an analysis of variance is carried out on the differences in scores from pre-drug baselines between the drug treatment and saline control groups. The averaged data are then transferred to the Time-Dose-Response Summary form shown in Fig. 4 where they are arranged in logical sequence for review and to reveal the dose-

response effects of treatment for each interval of measurement. As a visual aid, bar graphs are used to enclose the statistically significant treatment effects (solid black lines) and those not statistically significant which represent trend changes considered of probable therapeutic significance (dashed lines)[5,6].

Data Assessment and Interpretation

In assessing the data one should first scan the "0" time baseline and saline time-response results to get a feeling for the baseline state of the animals, inter-group variability, and to note any widely divergent responses. The latter should be checked as possible errors in *processing*, not just viewed as unusual group responses. Such scanning will reveal alley progression and locomotor activity scores to be fairly variable, and motor-affective responses (not transfer-arousal or touch-escape) to be somewhat more variable than the remaining data. The motor-affective responses are largely learned alternative modes of responding to provoking situations and stimuli, e.g., avoidance, struggle, escape, freezing, approach, etc., while the remaining measures are more physiologic and reflexive in nature and, accordingly, less prone to be variable. Locomotor activity and alley progression scores, also, tend to diminish as a function of time or repeated testing, i.e., they exhibit experimental "extinction." Drug effects on this phenomenon should be noted; the extinction is blocked by sedative-hypnotics, minor tranquilizers and amphetamine-type stimulants. One may also note a diminution of the finger-approach response when present, perhaps because of the aversive quality of the testing. This latter extinction can also be used to advantage; it enables one to better reveal any drug-induced enhancement of the behavior.

One next proceeds to discern the significant areas and patterns of change revealed by the bar-graphs, the doses at which they occur, and the relative selectivity of the changes of interest. In doing this it seems best to focus first on *transfer arousal, visual placing* and *bizarre behavior* scores, since these denote the state of behavioral arousal present and conscious awareness of the environment respectively, i.e., the capacity

[5] The bar-graph profile of the drug is intended to direct the attention of the viewer to changes of deemed *therapeutic* significance. The arbitrary requirements for statistical significance at the $p = < 0.05$ level is sometimes difficult to achieve with 6 animals per treatment group. The statistical tests do not take dose-trend effects or tangential supportive evidence into account.

[6] It would seem possible to integrate, further condense and summarize the accumulated data into some 15 logically consistent categories, e.g., as with struggle-escape behavior (touch-escape, positional struggle, startle and finger-withdrawal), sensoro-motor responses (pinna, corneal, toe- and tail-pinch), or muscle tone (grip strength plus abdominal, body and limb tone). Such processing may simplify inter- and intra-class drug comparisons, but is unlikely to serve as a substitute for the discrete data developed. It has not yet been undertaken.

of the organism to apprise the environment and cope with it. One should also note the *total incapacity* score, which indicates the capacity for motor performance. All depressant drugs, particularly the major tranquilizers (but also imipramine-type antidepressants) depress the arousal and placing responses; amphetamine-type stimulants increase transfer arousal scores but diminish visual placing (see below).

One next observes treatment effects on the pattern of motor-affective (interactional) responses to provoking stimuli, which can be interpreted in part to reflect drug effects on "social behavior," i.e., the interaction between animal and observer[7]. The data also serve to provide information on the pattern of "operant" behavior of the animals resulting from life-conditioning processes (rather than instrumental conditioning) and are differentially affected by drugs. The *touch-escape*, *positional struggle* and *finger-withdrawal* responses may be viewed as avoidance-escape type behavior; they are most prominently reduced by the major tranquilizers and increased by amphetamine-type stimulants. The *grasp-irritability* and *provoked biting* scores denote effects on fighting behavior; they are reduced by major and minor tranquilizers and increased by amphetamine type stimulants. *Provoked freezing*, an "active" immobility, seems increased by narcotic analgesics and decreased by minor tranquilizers while *positional passivity*, a "passive" immobility, is mainly increased by sedative-hypnotics and narcotic analgesics and reduced by amphetamine-type stimulants. The *finger-approach* response seems part of an approach-withdrawal conflict situation comparable to the Geller-Conflict Procedure (GELLER and SEIFTER, 1960) and is most prominently increased by the minor tranquilizers. Increased *vocalization* and *urination-defecation* scores generally, but not always, denote a state of heightened fearfulness or emotionality.

Sensoro-motor responses are a corollary to motor-affective responses and should be viewed next. They also reflect the capacity of the organism to respond to environmental stimulation, though of discrete sensory modality. They denote the extent to which the organism can respond to various types of sensory input and are of value in discriminating the various classes of drugs. The narcotic analgesics greatly reduce sensoro-

[7] In the broader sense, the interactional behavior of animals or humans includes three types—interaction with *inanimate objects* in the environment, with *animate objects* or with *oneself*. The modes of behavior expressed toward animate objects, e.g., approach, avoidance, biting, play, escape, grooming or eating behavior, are also expressed symbolically or actually toward physical objects or oneself. (The "meaning" of the object-stimulus alone determines the nature and magnitude of the response.) The effects of drugs on the specific motor-affective components of interactional response, therefore, can be expected to have generality to other objects of interaction, i.e., to vary only with the intensity of the sensory feedback stimulus provoked by the object.

motor responses (visual placing, tail-pinch, toe-pinch, pinna and corneal), particularly to a pain stimulus. Chlorpromazine-type major tranquilizers moderately reduce sensoro-motor responses while those of the reserpine-type do not (single dose). Minor tranquilizers reduce startle and increase pinna and corneal responses, while amphetamine-type stimulants increase the startle response.

Interpretation of the neurologic and autonomic measures would not seem to require much discussion; their meaning is more explicit. When associated with psychoactive drugs, however, major changes in these areas usually denote untoward side effects[8], and they are of value for discriminating certain drug types. For example, the triad of positional passivity, impaired righting and ataxia with similar scores and steep dose-response slopes, almost invariably denotes a sedative-hypnotic of the barbiturate type. The presence of miosis, lacrimation, blephorospasm and the absence of impaired righting can be used to distinguish reserpine from chlorpromazine-type major tranquilizers, while markedly increased tail elevation (Straub tail), duck-walk gait, mydriasis and muscle rigidity are diagnostic of narcotic-analgesics (tail elevation and duck-walk gait in the absence of mydriasis and muscle rigidity suggest narcotic antagonists).

Important to consider is that no single measure can be invariably diagnostic of the drug type, or have implicit meaning without reference to the other associated changes produced. Classification or discrimination of within-class differences requires emphasis on certain key measures within a wider configuration of changes associated with the drug action (a configurational approach). However, one is required to go beyond the configurations and terms used into an analysis of what one is actually measuring (or is available for measurement) with the operations carried out; i.e., to employ a "system analysis" approach (IRWIN, 1968). This type of approach has been applied in constructing the mouse observational procedure, where every effort has been made to provide the ancillary information required to interpret the meaning of a datum rendered. For example, the visual placing response is impaired by phenothiazine-type tranquilizers and amphetamine-type stimulants. With the former, the animal is depressed and less responsive to environmental cues; with the latter, the animal is more prone to turn and twist during the maneuver so as to inadequately monitor the situation. A similar reponse occurs when testing for the pinna and corneal responses. By introducing the measure "positional struggle", rated on the basis of the struggle behavior exhibited during these maneuvers, information not only is obtained on

[8] Small changes in neurologic and autonomic function are characteristically produced by altered states of excitability or behavioral arousal, as with muscle tone, respiratory rate or palpebral closure.

still another important measure, but more appropriate interpretation of the meaning of any observed changes in visual placing, pinna and corneal responses is made. Similarly, one can discern any of several responses when one provokes an animal by pinching its tail, e.g., escape, vocalization, freezing or biting. The tail-pinch response is scored on the basis of the magnitude of the motor response exhibited, but a reduced score does not necessarily mean "analgesia"; it may merely reflect a shift to another mode of responding which invites a lower score, e.g., freezing. Since the tail-pinch stimulus offers the possibility for measuring drug effects on the specific modes of response as well, these have been incorporated in the procedure and afford the possibility for more appropriately interpreting the meaning of the tail-pinch score. Through taking advantage of such multiple responses present in almost every situation, it has been possible to obtain a wider range of information with relatively few maneuvers.

It is within this broader framework of "system analysis" that the mouse observational procedure offers new possibilities. It provides a means for rapidly assessing and describing the behavioral and physiologic state of the organism, with major emphasis on those target functions of behavior which collectively express the temperament and behavior of the organism and delimit its capacity to respond to or cope with its environment. For specific drug or behavior research, the procedure provides the collateral information important for interpreting the significance of an observed change. For pre-clinical drug evaluation, it enables one to observe in animals effects similar to those the physician observes in humans and to perceive the quantitative time-dose-response relationships for the various effects within a *common system of measurement* (species, individual, environment, observer, procedure, handling, drug preparation, route of administration, day, and time of measurement). This condition is impossible to achieve through the combination of separately derived data, each from different systems of measurement.

Results

A number of factors that contribute to data variability were examined, including intra-observer differences, inter-observer scoring and animal handling differences, and variability due to changing physiologic responsiveness of the animals themselves.

Inter- and Intra- Observer Differences. Inter-observer differences in scoring were tested with three observers. One processed the animals after which all scored the behavior and responses independently. With occasional exceptions, when viewing the same event it was found possible to score the animal behavior and performance within the limits of 1.0 units of measurement on a 0 to 8 rating scale. This level of deviance

(12.5 percent) of scores between observers was considered pragmatically acceptable. Several classes of drugs and a wide range of dosage were employed for the testing, e.g., chlorpromazine hydrochloride, meperidine hydrochloride, imipramine hydrochloride and pentobarbital sodium.

Inter-observer variability due to differences in animal handling (as well as scoring) was determined from the results of three separate drug studies carried out simultaneously, but independently, by two observers on a blind, randomized treatment basis. Each observer contributed half of the data. The studies were intra-class comparisons respectively of tricyclic antidepressants (imipramine, desipramine, amitriptyline and nortriptyline; 12 mice per dose), hypnotics (pentobarbital, glutethimide, chloral hydrate and ethyl alcohol; 6 mice per dose), and narcotic analgesics (morphine, methadone, meperidine and phenazocine; 6 mice per dose). Each study included 24 treatments orally administered per test day, i.e., 4 drugs, 5 dose levels of each, and 4 saline control groups. To increase the N per observer for the analysis of observer differences, the time-response data from each study (4 drugs and 5 intervals of measurement, e.g., 0-, 15-, 45-, and 150-min post-drug) were combined for each dose level of drug tested. This provided a sample size of 60 for the hypnotic and narcotic analgesic studies per dose level per observer, and a sample size of 120 per dose level per observer for the tricyclic antidepressant study.

The results, illustrated in Fig. 5, show the magnitudes of the difference of mean scores between observers for each dose level of treatment and the means of the combined doses. In the figure, all inter-observer differences exceeding the pragmatic level of significance sought (12.5 percent of the maximum score possible) are heavily outlined. They were presumed to reflect mainly the contribution to variability of investigator differences in animal handling. The differences were greatest for the tail-pinch, alley progression, and visual placing response, but were not uniformly distributed across the several studies and in no instance exceeded 25 percent of the maximum score possible. In isolated instances, as for ataxic gait and total incapacity, inter-observer differences increased with rising dosage, but even this was not uniformly observed. As may be noted from the signs in Fig. 5 (e.g., — or +), each observer generally scored consistently higher or lower than the other observer. This suggests good intra-observer consistency and data reliability.

Test-Retest Reliability. Test-retest reliability seemed as much contingent on the physiologic responsiveness of animals to drugs (including seasonal and climatic factors) as on differences in animal handling and scoring. The data for this analysis were derived from a study comparing the effects of imipramine, chlordiazepoxide and meperidine on 11 strains of mice, one of which (Berkeley Swiss, female, 6 weeks old) was replicated

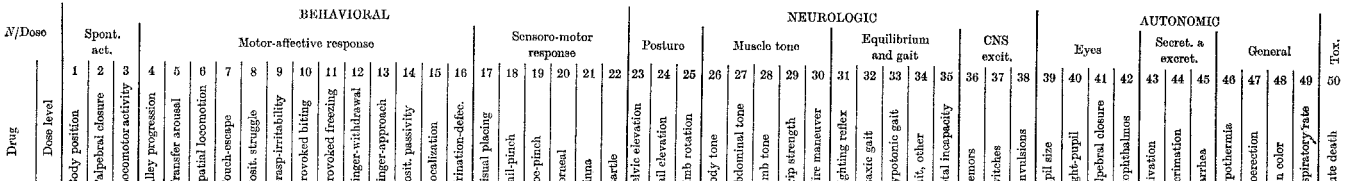


Fig. 5. *Inter-observer differences in scoring.* The figure denotes the magnitude of the differences in scoring between observers derived from three studies with tricyclic antidepressants, hypnotics and narcotic analgesics respectively. Differences exceeding 12.5 percent of the maximum score possible, e.g., 1.0 units on a 0 to 8 scale, are enclosed by heavy black lines and are presumed to arise mainly from differences in animal handling. None of the differences exceeded 25 percent of the maximum score possible

Fig. 6. *Test-retest variability in response to imipramine.* The figure shows the averaged data of ten replicated studies with saline and imipramine at approximately weekly intervals. During this period, the animals exhibited gradually increased sensitivity to certain of the drug effects (designated by the symbol "+"). Circled numbers denote differences from the group mean greater than 12.5 percent of the maximum score possible. The latter seem largely attributable to animal rather than observer imposed variability

with each of the other strains (10 times) as a standard of reference. Physiologic saline and 2 dose levels of each drug were administered orally on a blind, randomized basis. Two observers were employed, each contributing half the data. Fig. 6 shows the mean responses obtained 60 min after the administration of saline (12 mice per group) and 2 dose levels of imipramine (6 mice per group), the results being representative of those also obtained with chlordiazepoxide and meperidine.

The 10 replications with saline during a two-month period revealed a very slight trend toward diminished limb tone and increased hypotonic gait, as indicated by the symbol "+". During this same period, the drug-treated animals exhibited significantly increased responsiveness to many of the drug actions. This also was noted with the chlordiazepoxide and meperidine studies. It could not be attributed to systematic changes in observer scoring, as the trend changes were not evident across the other strains of mice tested (except for the scoring of hypothermia).

The circled numbers in Fig. 6 denote all values which deviated from the mean of the 10 replications by 12.5 percent or more of the maximum rating score possible, by 1.0 units or more for vocalization and urination-defecation frequencies, and by 10 cm or more for the alley progression scores respectively. Deviations of this magnitude were rare among the saline-treated animals. They were more prominent following drug administration, increased with the dose of drug administered, and seemed largely due to animal differences in responsiveness to drugs rather than to observer differences in animal handling or scoring.

In general, the reliability studies revealed a high level of inter-observer reliability when viewing the same event, good internal intra-observer consistency in scoring on retesting, but significant inter-observer differences in animal handling and processing that modified the response to treatment so that one observer consistently scored somewhat higher or lower than the other. Finally, animals were noted to undergo systematic changes in physiologic responsiveness to drugs with changing season, contributing a source of variability unrelated to the performance of the observer. The data revealed inter- and intra-observer reliability well within the pragmatic requirements for scientific study, and a level of test-retest reliability sufficient to identify and differentiate different classes of pharmacologic agents and their members. These studies merely reconfirm that the response to drug is entirely contingent upon the system of measurement employed, i.e., that it represents a drug-tissue-subject-environment-observer interaction.

Response Profile of Chlorpromazine

The time-response profile of orally administered chlorpromazine hydrochloride was assessed on a blind, randomized basis (as described above) by two observers, each contributing half the data (total of 6 animals per dose tested). An analysis of variance was performed to determine the drug-induced changes statistically significant from saline controls at the $p < 0.05$ level. Fig. 4 shows the means of all the data arranged on a time-dose response axis. The solid-line bar graphs enclose

the statistically significant data; the interrupted-line bar graphs enclose the changes of probable biologic but not statistical significance for the number of animals tested.

The data reveal slight palpebral closure (α-adrenergic blockade) and reduced behavioral arousal, touch-escape, visual placing (awareness), biting, tail elevation and muscle tone after 1 and 3 mg/kg; marked reduction of these functions with hypothermia and markedly impaired hypotonic gait after 10 mg/kg; extreme effects with prostration after 30 mg/kg; and delayed mortality after 100 mg/kg. Untoward autonomic and neurologic side effects were thus evident at the lowest effective behavioral dose. Peak effects occurred after one hour and the duration of action was 8 to 24 hours, depending on the dosage.

Discussion

Success in drug evaluation and the prediction of drug effects from animals to man requires a baseline of relevant, quantitative information; the more related and directly referential to the human condition the information is, the more easily and accurately is prediction likely to proceed. It is implicit that the methods used be sufficiently sensitive to detect the therapeutic-equivalent effects sought and deemed acceptable for humans (IRWIN, 1966). The mouse procedure described fulfills this criterion and, in addition, detects such effects at doses comparable to those employed for humans, e.g., 0.3 mg/kg of methamphetamine and 1.0 mg/kg of chlordiazepoxide or chlorpromazine. Subsequent papers will show the results of application of the procedure.

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