An evaluation of carbon dioxide as a short acting anesthetic

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The use of carbon dioxide (CO_2) as a quick-acting anesthetic of short duration was studied. Sixteen rats were exposed to a CO_2 atmosphere for 10 and 15 sec on separate days. Records were taken of the time required for the administration of intramuscular and gavage injections as well as recovery of motor functions. Recovery following both exposure times was less than 2 min. Data are presented that show that there are no prograde effects of CO_2 exposure on subsequent variable-interval (VI-15 sec) behavior.

Many investigators have utilized the technique of stomach loading in order to benefit from the advantage of slow drug absorption yielding long-duration drug effectiveness. One method of stomach loading involves the gavage injection that entails the insertion of a blunt tube down the esophagus and into the stomach. An inexperienced person frequently traumatizes the animal, producing a rather vigorous emotional response consisting of a high degree of motor activity, such as clawing and biting. This behavior, whether precipitated by the experienced or inexperienced person, makes it difficult for a single E to perform a gavage injection with any degree of assurance that tissue damage will not occur. On occasion, the severity of damage is so great that the animal is rendered useless. Any harmful physical effects would preclude the use of gavage injections in a steady-state experiment, where the health of the animal is so critical to continued investigation.

A second source of difficulty arises when the procedure differentially affects an animal's baseline level of responding. Even when such a response is infrequent, its occurrence provides an additional source of variability in many behavioral measures.

An extremely short-acting central nervous system depressant serves to minimize the likelihood of tissue damage and affords some uniformity of treatment. Gaseous carbon dioxide (CO_2) tends to depress the central nervous sytem (Dunlop, 1957) and has been used as a quick-acting, short-duration anesthetic for insects (Williams, 1946). Murkland (1967) used CO_2 to anesthetize rats prior to a gavage injection of magnesium pemoline.[1] Submersion of the rats in an atmosphere of near-100% CO_2 for 25 sec was sufficient to allow the E to employ easily the gavage injection, with recovery of motor functions approximately 1 min after the injection.

Numerous investigators have used CO_2 to produce hypoxia and have found that such an exposure will increase escape behavior made contingent upon reduction of the CO_2 concentration (Weinstein, 1966). Exposure to CO_2 will also reduce locomotor behavior (Gerben, 1968) and has been used to produce retrograde amnesia in rats (Quinton, 1966) and in cockroaches (Freckleton & Wahlsten, 1968). Although CO_2 has been demonstrated to act in part as an aversive stimulus (Leukel & Quinton, 1964), recent research by Quinton (1966) has shown that it possesses both amnesic and aversive qualities. The aversiveness becomes pronounced only with massed trials treatment.

In view of these findings, it appears that CO_2 provides an extremely short-acting anesthetic with a rapid onset that would be useful when gavage injecting an animal. In addition, the procedure might be beneficial to a single investigator who had simultaneously restrained and injected an animal with either the gavage or intramuscular (IM) method. A primary problem is that the amnesic and aversive properties of CO_2 might maintain behaviors that would disrupt the animal's baseline behavior.

The present investigation was conducted to show that short exposure to an atmosphere of CO_2 would allow sufficient time for gavage or IM injections and quick recovery from the anesthesia. Data are presented to show that experimental behavior is unaltered even when the anesthetic is administered immediately prior to introduction of the experimental conditions.

METHOD

Subjects

Sixteen male albino rats of the Sprague-Dawley strain, ranging in weight from 292 to 506 g, were used.

Apparatus

A 2000-psi cylinder of carbon dioxide (CO_2), fitted with a regulating valve, was connected to a 6¾ x 4¾ x 12½ in. glass battery jar with rubber tubing. A Plexiglas lid over the battery jar had two 1-in. openings in opposite corners. The tubing from the CO_2 tank entered one of these holes and connected to ¼-in.-i.d. glass tubing that projected to 1 in. above the glass bottom. The jar had a false floor made of ½-in. hardware cloth that was elevated 1½ in. above the glass bottom. This arrangement, in effect, allowed the CO_2 to enter below the floor and exit at the top of the jar.

Procedure

The Ss were divided into two groups receiving either 10 or 15 sec of CO_2 exposure, according to body weight so that the mean weights and ranges of the two groups were similar. These groups were further subdivided for treatment in such a way that order effects were controlled. On the first test day, each animal was brought into the testing room. The battery jar was filled with CO_2 under 100-lb pressure for 30 sec. By checking the atmosphere for oxygen concentration with a Beckman O_2 analyzer, 30 sec was found to be somewhat beyond the time required for zero oxygen in the jar.

The valve was then turned off and the animal placed in the CO_2 atmosphere for either 10 or 15 sec. At the end of this interval, the S was quickly removed and received either an IM or gavage injection of 0.2 cc isotonic saline. Following the injection, the animal was placed on a table and recordings of the times required for injection, return of eye color, and movement were made.

On the second test day, all Ss that had previously received a 10-sec exposure were placed in the chamber for 15 sec, and vice-versa. Also, those animals receiving one type of injection on Day 1 received the opposite on Day 2. All measures and procedures were identical on both test days and involved a total time of approximately 3½ min from the filling of the CO_2 tank to returning of the animal to his home cage.

RESULTS AND DISCUSSION

Several duration measures were taken to evaluate the procedure of anesthetic administration, saline injection, and recovery from anesthesia. Two of the three critical measures were the times necessary for (1) S to move his fore-paws, and (2) color to return to S's eyes after removal from the CO_2 atmosphere. The third measure was injection duration. Table 1 contains the means and standard errors of the mean for each group on each test day. There was no systematic difference between the 10- and 15-sec

Table 1
Means and Standard Errors for Recovery Times in Seconds for Both 10 and 15 Second Exposures and for Intramuscular (IM) and Gavage Injections (GI)

	Day 1								Day 2							
	10				15				10				15			
Measures	IM		GI		IM		GI		IM		GI		IM		GI	
	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE
Length of Operation	4.25	.05	7.00	1.53	8.00	2.38	11.70	3.74	4.38	.56	9.50	1.76	6.75	.75	7.00	.41
Color Return	37.50	2.63	31.00	1.00	17.75	.86	44.50	3.79	26.00	3.24	21.67	2.68	39.50	2.46	37.75	2.02
Movement	60.50	4.83	56.00	6.75	70.00	5.97	79.50	4.22	46.30	12.02	44.33	8.98	62.00	4.49	61.25	6.89

exposure groups on either the first or second measures, nor was there a difference between the gavage- and IM-injected animals. This lack of a systematic difference is reflected by the fact that on Day 1 the 10- and 15-sec IM groups took, respectively, 60.5 and 70.0 sec to recover movement; on Day 2, the times were 62.0 and 46.3 sec.

Two additional Ss were run in an operant situation where each received reinforcement for bar pressing on the average of once every 15 sec on a variable-interval 15-sec (VI-15 sec) schedule. Ss R1 and R2 received 10- and 15-sec exposures to CO_2 under the same conditions as those Ss previously mentioned. The data for these two Ss are shown in Fig. 1. Cumulative Record A for each S is representative of performance under the VI-15 sec schedule prior to CO_2 exposure. Cumulative Record B shows the same schedule after CO_2 exposure. Reinforcements are shown as pip marks along the event line.

The overall rate for R1 on the control session was 16.5 responses per minute as compared to a rate of 15.9 on the treatment session. A comparison of Records A and B for R1 shows very slight local rate differences. The only apparent difference is a slightly lower rate for the middle portion of the treatment session relative to the same portion for the control session. However, the rate late in the control session is slightly lower than the rate during the same segment of the treatment session. The overall rate for R2 on the control session was 19.8

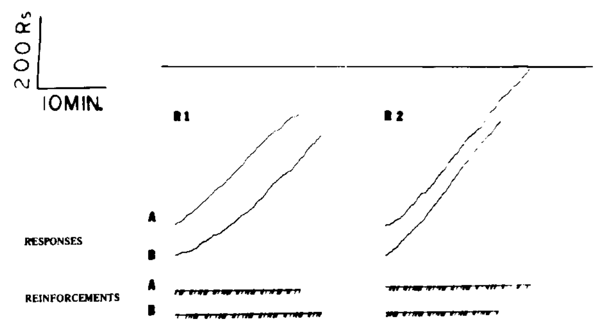

Fig. 1. Selected cumulative response records of performance on a VI-15 sec schedule of reinforcement for R1 and R2 during a control session and an experimental session. Cumulative responses are represented in the upper half of the figure and reinforcements are indicated by the pip marks on the lines below. Responses and reinforcements during the control session are indicated by the portions of the figure labeled "A." The portions of the figure labeled "B" indicate responses and reinforcements during the experimental session. The time response scale in the upper left-hand corner provides an indicant of rate per minute, i.e., a diagonal line that equally divides the area between the axes represents a response rate of 20 responses per minute.

responses per minute as compared to 22.8 following treatment. Even though R2 received a 15-sec exposure to CO_2, the differences between Records A and B are slight. Since these Ss were placed in the operant chamber no more than 5 min after exposure to CO_2, the data adequately demonstrate that the residual effect of the anesthesia is either very short term or nonexistent.

Of the 16 animals anesthetized and then injected in the first part of this study, none demonstrated obvious emotional behavior during the injections; therefore, the likelihood of physical harm during the injections was greatly reduced. Furthermore, these animals were checked at 2, 10, and 20 days following the injection and their weights were almost identical to the pretreatment weights. The behavioral data in Fig. 1 indicate that even intervals as short as 5 min following CO_2 anesthesia show no carry-over effect into the experimental session.

The claim could be made that subjection to CO_2 anesthesia for even a 15-sec duration might make the animal more receptive to viral attack so that pneumonia might be more easily acquired. Twenty days following treatment, a visual and stethoscopic check of each animal's nasal cavities and respiratory system, respectively, indicated that none of the animals had acquired such a disorder. Generally, we have concluded that this type of anesthesia may be used when animals are subjected to painful injections, stomach loading of capsules or other foreign matter, and other painful manipulations, e.g., attachment of electrodes and cannulae, or ear-marking with an ear punch. Also, the likelihood of the E's receiving a humiliating and painful rat bite is minimized.

Such retrograde effects of CO_2 as amnesia and aversiveness are generally irrelevant with this technique; the only concern lies with possible prograde effects. From the behavior sampled in this experiment, it is clear that possible prograde effects of two exposures of CO_2 may not be serious. However, these claims cannot be made for the chronic use of CO_2, or for behaviors other than VI responding. The major advantage of the procedure lies in the fact that all animals receive markedly similar stimulation and can receive treatment by only one E. This would be difficult with unanesthetized animals. Furthermore, the temporal effects of this method of anesthesia appear shorter than with other more common techniques.

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NOTE

1. We are indebted to Mr. Robert Murkland for providing pilot data which led to the present investigation.

A wide range timing circuit with two independent controls

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The timer described in this note is an example of how a small increase in engineering complexity can lead to a marked increase in user convenience and overall system accuracy.

The timer was designed for an experiment by Owens (1969) where the S dropped marbles into a box, competing against a machine dropping marbles into another box. The timing circuit had to produce pulses at a base rate which could be set for each individual S. Each pulse caused the machine to drop one marble. During the experiment, trials were run at this base rate and at fixed percentages above and below the S's base rate. While this can be done with any timer that has a calibrated dial, such an apparently simple system gives considerable work for the E. It also suffers from the errors of any system where repeatability depends on the accuracy with which an E can reset a dial. With only a small increase in engineering complexity, these difficulties are overcome, resulting in a device that is ergonomically sound, and has worked without trouble for some 200 Ss in Owens's experiment.

The circuit is shown in Fig. 1. It is based on a recently available device, the GE programmable unijunction transistor, D13T2, and circuits for the same, described by Spofford (1967).

The D13T2 used in this oscillator has the advantage over the more common unijunction transistor of providing two independent places in the circuit where, by simple resistive potentiometers, the time of the oscillator may be varied.

Q1, C1, R1-R12, and D1 form a free-running relaxation oscillator. The base rate is determined by the setting of R1 in conjunction with R2 and C1. The fixed percentage increase and decrease from base rate is determined by the potential at R4. This potential is dependent on the setting of Switch S1. In the mid-position (base rate), R4 is connected to a fixed voltage determined by R8 and R9. In the up position (faster rate), the voltage at R4 is lower and determined by the setting of R6 and the values of R5 and R7. In the down position (slower rate), the potential at R4 is higher, being determined in a like manner by R10, R11, and R12.

The percentage increase or decrease of the oscillator frequency obtained from the three positions of the fast-normal-slow switch, S1, is independent of the setting of the base rate control, R1.

The oscillator is coupled via C2 to a one-shot circuit, Q2 and Q3 and associated components, that drives the solenoid, L1, through an inverter, Q5. An operating pulse of 40 msec was delivered to the solenoid at each cycle of the oscillator, and this allowed one marble to be dropped by the apparatus. The power supply is unregulated for the solenoid supply and Zener-diode regulated for the 12-V supply.

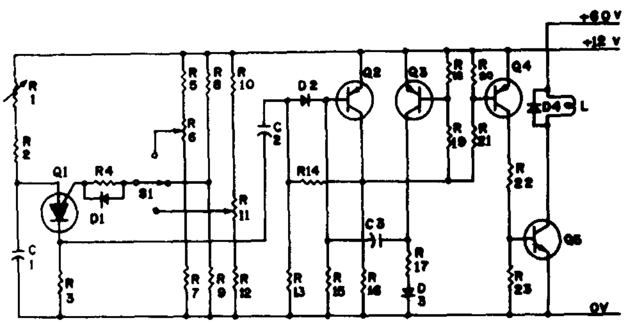

Fig. 1. Circuit diagram and parts list for timing circuit: R1–1M variable; R2–330K; R3–100 ohms; R4–1M; R5, 8, 9, 12, and 14–4.7K; R6 and 11–2.5K variable; R7, 10, 22, and 23–2.2K; R13–33K; R15, 19, and 21–6.8K; R16, 17, 18, and 20–1K; C1–2 mfd mylar or better; C2–0.02 mfd mylar; C3–6 mfd 15 V electrolytic; D1, 2, 3, and 4–1N4154 or similar; L1–500 ohms solenoid; Q1–D13T2; Q2, 3, and 4–2N1305 or similar; Q5–2N3440 or similar; S1–3-position single-pole switch. All fixed resistors ½ W ±10% carbon composition, variable resistors. Ohmite Type AB or equal. For greater stability use metal film, deposited carbon, cermet, or wirewound precision resistors, and a polycarbonate or polystyrene capacitor for C1.

The oscillator timing stability depends primarily on the quality of C1 and R1 and R2. For C2, a mylar capacitor is suitable as it has low leakage and low temperature coefficient. Polystyrene and polycarbonate capacitors are better but more expensive. The resistors can all be carbon composition for most applications. Precision metal film, deposited carbon, or cermet resistors all have lower temperature coefficients and higher long-term stability. The timing stability of the oscillator is almost independent of the supply voltage (a 10% charge in supply voltage gives less than ½% charge in timing). Using a mylar capacitor for C1 and carbon resistors in a normal room temperature, the short-term stability can be expected to be within ±1%.

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