## Experimental Morphine Addiction: Method for Automatic Intravenous Injections in Unrestrained Rats

*Abstract.* An operant behavioral study on morphine addiction utilized a self-injection technique for giving intravenous injections to relatively unrestrained rats. The rate of self-injection varied inversely with the dose. Morphine was a reinforcer that produced almost immediate satiation.

Operant conditioning has been applied in recent years to the study of the effect of drugs on animal behavior. This report describes a method for using drug administration as a positive reinforcer of a lever-press response in rats. It was presumed that such operant behavioral studies would require relatively unrestrained animals and intravenous injections to minimize the time between lever press and drug effects. Many animal studies have demonstrated that repeated administration of opiates can lead to tolerance and physical dependence, as evidenced by an abstinence syndrome and a desire to continue opiate administration (*1*).

With the method I used, rats could move freely about their cages, carrying a lightweight saddle strapped behind the forelegs. The saddle was connected by a sprocket chain to a small swivel and stuffing box to permit injection of drugs. Intravenous injections were made through a polyethylene cannula passed down the jugular vein into the right heart. Cannulae could be expected to remain functional for at least several weeks (five of ten lasted 9 months), and differed from other rat heart cannulae (*2*) in two important details: the intravenous portion was drawn down to 0.2 to 0.4 mm outside diameter, and the exit through the skin was reinforced (*3*). Injections were by automatic syringe drivers or burettes whose output was programmed on film (*4*). Rats were albino females weighing 200 to 250 g.

Two series of experiments were conducted. In the first, physical dependence upon morphine sulfate was established by hourly doses increased in a 2.5-percent geometric progression from 2 to 40 mg/kg of base (122 doses), the last dose being repeated for 1 to 2 days. Next, a lever was put into the cage, which, when pressed, caused injection of 10 mg/kg of morphine as the sulfate. After experiencing drug effects a few times by chance lever pressing, rats responded regularly. After 2 days the dose was reduced to 3.2 mg/kg, whereupon the response rate promptly increased. Abrupt withdrawal by disconnecting the syringe led to a prompt increase in response rate (seven fold average) which lessened gradually after the first 3 hours (Table 1). Response rate increased before overt signs of abstinence appeared. Overnight withdrawal caused an abstinence syndrome of weight loss (about 20 percent), tremor, hypersensitivity, agitation, soft stools, and increased respiration, but the rats remained gentle.

In the second series, reinforcement ratio (responses per injection) was also varied. Physical dependence was established as before, except that the dose was increased to a maximum of only 20 mg/kg (94 doses). For the first 12 to 24 hours, each response injected 3.2 mg/kg; then for periods of 23 to 34 hours each the program was 5 : 1 at 3.2 mg/kg, next, 10 : 1 at 3.2 mg/kg, and finally 10 : 1 at 10 mg/kg. The change from continuous reinforcement to 5 : 1 decreased net morphine intake. Presumably, the delay enforced by extra responses allowed full effects of a dose

Table 1. Responses per hour for self-administered morphine by addict rats on continuous (1 : 1) reinforcement.

| Condition | | Responses per hour | | | | | | Daily intake (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| Reinforcement ratio | Dose (mg/kg) | Rat 95 | Rat 224 | Rat 255 | Rat 261 | Rat 402 | Av. | |
| 1:1 | 10 | 2.4 | 2.0 | 1.3 | 0.9 | 1.2 | 1.6 | 384 |
| 1:1 | 3.2 | 5.5 | 3.1 | 2.9 | 2.5 | 2.1 | 3.2 | 240 |
| Withdrawal* | | 33 | 11 | 21 | 27 | 17 | 22 | |

* Calculations on first 3 hours only.

Table 2. Responses per hour for self-administered morphine by addict rats on fixed-ratio reinforcement.

| Condition | | Responses per hour | | | | | Daily intake (mg/kg) |
|---|---|---|---|---|---|---|---|
| Reinforcement ratio | Dose (mg/kg) | Rat 470* | Rat 483 | Rat 485 | Rat 490 | Av. | |
| 1:1 | 3.2 | 1.8 | 1.1 | 1.9 | 3.5† | 2.6 | 160 |
| 5:1 | 3.2 | 7.4 | 4.4 | 7.1 | 5.2 | 6.0 | 91 |
| 10:1 | 3.2 | 13 | 7.6 | 12 | 12 | 11 | 84 |
| 10:1 | 10 | 5.4 | 4.7 | 5.2 | 4.7 | 5.0 | 120 |

* Illustrated in Fig. 1. † Records of only last 7½ hours of 24-hour period available.

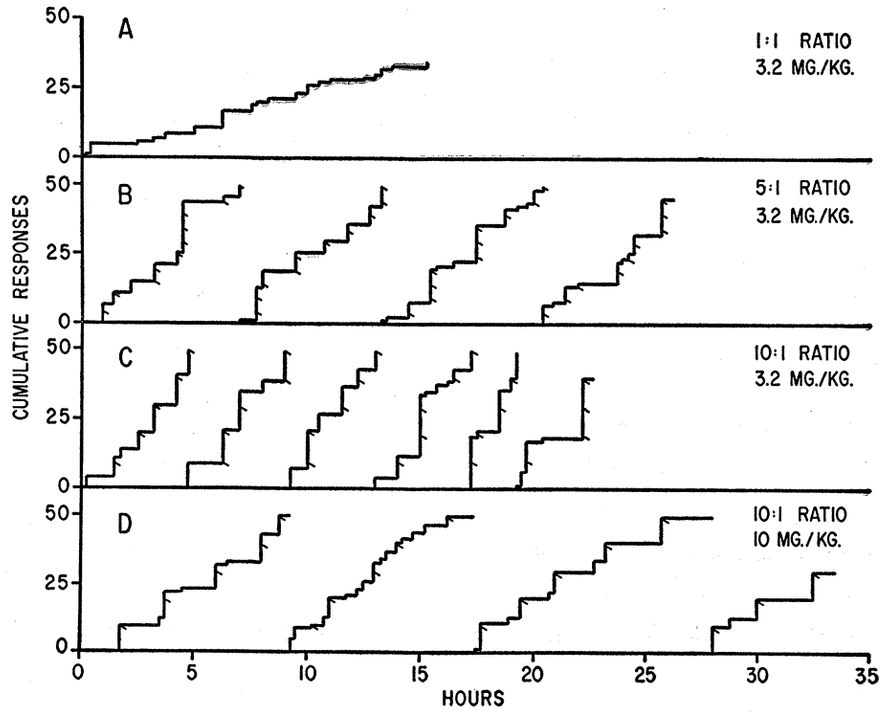


Fig. 1. Self-administered morphine by addict rat. Responses are plotted to the nearest 15 minutes, performance continuous. In *A*, there was one injection for each response. In *B*, *C*, and *D*, injections were at diagonal marks. For the last 15 hours in *D*, responses, in groups of 7 to 10, were terminated by an injection.

to be felt before a second was taken. When the ratio was increased from 5 : 1 to 10 : 1, response rate about doubled; when the dose was then increased to 10 mg/kg, the response rate decreased (Table 1). Performance of one rat is shown in Fig. 1.

Nalorphine, a morphine antagonist, temporarily induces an acute abstinence syndrome (*5*). These same four rats, when on a 10 : 1 ratio at 10 mg/kg, were given 4 mg/kg of nalorphine hydrochloride intraperitoneally. Within the next hour, three responded 50 times each, and the fourth, 20 times.

The most interesting aspect of ratio reinforcement was the response pattern after about 2 days on the same ratio and dose. Prolonged periods of no responding alternated with brief periods at a high rate terminated by an injection (Fig. 1*D*). This evidence indicates that the drug was a reinforcer that produced almost immediate satiation.

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#### References and Notes

1. H. Krueger *et al., Public Health Repts. (U.S.) Suppl.* **165**, 1 (1941); M. H. Seevers, *Ann. N.Y. Acad. Sci.* **51**, 98 (1948); S. D. S. Spragg, *Comp. Psychol. Monogr.* **15**, 1 (1940); J. R. Nichols *et al., J. Pharm. Sci.* **45**, 788 (1956); ——— and W. M. Davis, *ibid.* **48**, 259 (1959); H. D. Beach, *Can. J. Psychol.* **11**, 104 (1957); A. Wikler *et al., Federation Proc.* **19**, 22 (1960).
2. V. Popovic and P. Popovic, *J. Appl. Physiol.* **15**, 727 (1960); M. A. Slusher and B. Browning, *Am. J. Physiol.* **200**, 1032 (1961).
3. Details of fabrication and the use of cannula, saddle, and swivel will be included with my reprints, and have also been deposited as Document No. 7304 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D.C. Either a photoprint or 35-mm microfilm copy may be secured by citing the document number and making an advance payment of $1.25 to Photoduplication Service, Library of Congress.
4. Construction details of programmed syringe driver and automatic burette control are available on specific request.
5. L. A. Woods, *Pharmacol. Rev.* **8**, 175 (1956).

12 April 1962

## Method for Continuous Infusion of Fluids into the Chorioallantoic Circulation of the Chick Embryo

*Abstract.* A method is described for maintaining long term infusions of fluids into the chorioallantoic circulation of the embryo of the domestic fowl. Infusions can be continued up to 4 days in 6- to 17-day-old chicks, with subsequent hatching.

In order to evaluate the effect of certain substances upon the developing embryo it is often necessary to maintain an effective circulating level during a prolonged, readily controlled interval of time. We have devised a simple method of chronic infusion into the chorioallantoic circulation of the chick embryo. It has been used successfully for the infusion of curare and other neuromuscular blocking agents; pilot studies are now underway utilizing a variety of active substances. Because of the potentially broad applications of this method in embryological research, it is reported here.

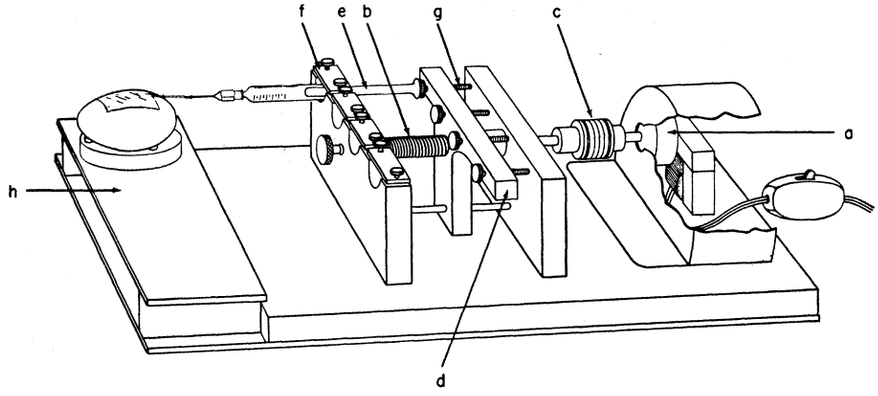


Fig. 1. Diagram of infusion pump in operation.

Essentially, the apparatus consists of a motor-powered multiple syringe drive and glass syringes, coupled to specially shaped microcatheters.

The syringe drive mechanism (Fig. 1) is adapted after Singer (*1, 2*). A synchronous motor (*a*) rotates a drive screw (*b*) coupled via a sylphon bellows (*c*), thus advancing a horizontal bar (*d*) which slowly depresses the syringe plungers (*e*). The syringe barrels are firmly held by clamps (*f*). Plunger adjustment screws (*g*) and an egg platform (*h*) are provided. An infusion rate of approximately 0.01 ml/hr has proved successful with 6- to 17-day-old embryos. We have achieved this rate by using a Telechron 1-rev/hr motor, a drive screw with 40 threads per inch and a 0.5-cm$^3$ Becton Dickinson tuberculin-type syringe. The rate can be varied by substituting different motors, drive screws, or syringes. Calibration is easily accomplished by allowing the apparatus to run for a given period of time and observing the distance traversed by the syringe plungers.

To prevent leakage of fluid during operation, the syringe plungers and hubs must be coated with high-vacuum grease.

The microcatheter (Fig. 2) is fashioned by hand from No. 10 or No. 20 polyethylene tubing. With moderate practice it can be made in about 3 minutes. The polyethylene is first drawn to a fine taper over a microflame. Two right-angle bends are made by touching the tapered tubing to a warm Nichrome wire controlled by a rheostat. The shaft of the catheter is straightened by contact with the Nichrome heating element. The tip is beveled with a sharp razor blade. Finally, the butt end is flared over a small flame.

Because of certain characteristics of the chorioallantoic circulation, the dimensions of the catheter must be tailored to the age of the embryo to be infused. The chorioallantoic membrane first appears on the 5th day of incubation (*3*). Its blood vessels are sufficiently sturdy to withstand infusion from the 6th day to the 18th or 19th day, at which time the membrane begins to dry out preparatory to hatching. With increasing incubation age, the membrane floats lower beneath the shell and the vessel walls increase in thickness. Thus, the catheter's tip diameter varies from about 200 to 325 $\mu$, the shank length varies from 4 to 8 mm, and the tip length varies from 2.5 to 4 mm at the extremes of the ages used.

The syringe is greased and filled with the injection fluid. A catheter is fitted to the needle tip. Care must be taken to exclude air from the system. The egg is candled, and a Y-shaped chorioallantoic venous (or arterial) bifurcation is located. A rectangular window is removed from the shell and inner shell membrane overlying the selected area. The egg is placed under a dissecting microscope, with the syringe-catheter assembly supported so that the tip overlies the stem of the Y-shaped bifurca-

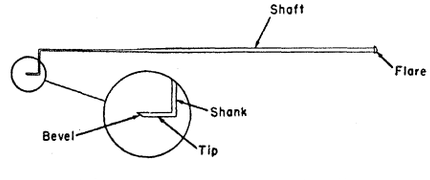


Fig. 2. Microcatheter (detail).