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BRIEF COMMUNICATION

Acute Intravenous Infusion in Freely Moving Rats Through the Sagittal and Transverse Sinuses

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DAVIS, M. AND R. D'AQUILA. *Acute intravenous infusion in freely moving rats through the sagittal and transverse sinuses.* PHARMAC. BIOCHEM. BEHAV. 4(4) 469–472, 1976. – A technique is described which allows drugs to be injected intravenously using the junction of the sagittal and transverse sinuses as the point of entry into the venous system. The procedure is rapid and uses conventional stereotaxic techniques. Subsequent restraint of movement during drug infusion is minimal. Using this method, 15 μg/kg d-lysergic acid diethylamide produced an increase in acoustic startle amplitide within about 1–2 min which lasted for about 25 min.

Intravenous LSD Lysergic acid diethylamid Startle

ADMINISTRATION of drugs through chronic indwelling catheters in freely moving rats is often preferable to acute injection of drugs when ongoing behavior is being studied. With such systems drugs can be infused remotely, without handling, so that ongoing activity or performance of the animals at the time of injection is not disturbed. Chronic infusion systems also allow the study of self administration of drugs. The intravenous (IV) route compared to other routes of drug administration (e.g. intraperitoneal IP) allows the most precise estimate of both the time course of drug action and minimal dose required to alter behavior.

Presently, several catheterization techniques are available for chronic intravenous infusion in freely moving rats [1, 2, 3, 4, 5, 7, 8, 13, 14, 15, 16]. In all cases catheterization of either the external jugular or the tail vein has been used. Another prominent vascular space is the junction between the transverse and sagittal sinuses on the top of the brain. The sinus at this point is about 2 mm wide and lies directly below the junction of the transverse and sagittal sutures (lambda) on the top of the skull. This provides an accessible landmark for the location of the sinus and a rigid structure directly above it for anchoring a catheter. The superior sinuses are the main drainage routes of the brain and have a fast rate of blood flow. Although an exact measure in the rat has not been made, the rate of blood flow through this area is probably on the order of 0.9–1.2 ml/min for a 350 g rat. This estimate is based on the fact that the average rate of blood flow through the rat brain is about 1 ml/min/g of tissue [10], that the weight of the brain of a 350 g rat is about 1.5 g, and that the sagittal sinus carries about 60–80% of the total blood from the brain.

In view of the size, accessibility, and fast rate of blood flow in this area, we have developed a technique which uses the confluence of the transverse and sagittal sinuses as the point of entry into the venous system. Basically the method involves the implantation of a fine hypodermic needle into the junction of the sinuses and securing it with epoxy to a previously implanted guide tube. The surgery is rapid and uses conventional stereotaxic techniques. By using rates of infusion that are well below the average rate of blood flow through this area, local complications such as distention of the sinus or drugs backing up into the sagittal sinus can be avoided. To test the method, the effect of d-lysergic acid diethylamide (LSD) on the acoustic startle response was measured. Startle is a sensitive index for evaluating drug effects on behavior and is augmented by low doses of LSD [6,11].

PRELIMINARY EXPERIMENTS

To evaluate the feasibility of injecting drugs through the transverse-sagittal sinus junction, the following preliminary experiments were done. Rats were anesthetized with chloral hydrate (400 mg/kg, IP) and placed in a stereotaxic instrument. A flap of bone about 1 cm square centered over lambda was removed using a fine high speed circular grinding wheel (Moto Tool Cutting Wheel No. 409). By careful dissection, the bone flap can be removed with the dura largely intact so that the sinuses can clearly be seen (Fig. 1). A 27 ga hypodermic needle (regular point), connected via PE 50 intramedic tubing through a 2-way valve to a syringe and infusion pump, was then lowered

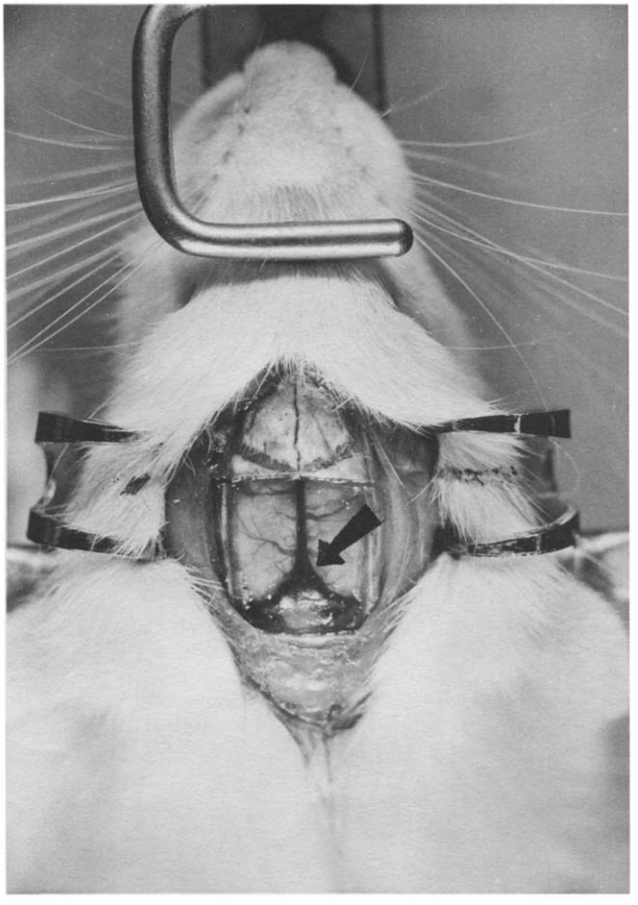

FIG. 1. Photograph of the top of a rat skull, with a 1 × 1 cm square of bone flap removed to show the sagittal (between the cerebral hemispheres) and transverse (between the cerebral hemispheres and cerebellum) sinuses. Arrow points to junction of the sinuses where hypodermic needle will be placed. Removal of the bone flap was only used in preliminary experiments and is not part of the actual method.

until it first dimpled the membranes about the sinus and then punctured and entered the sinus. When penetration had occurred a steady column of blood could readily be withdrawn and reinfused. Sterile saline could be injected at rates up to 4 ml/min with no detectable leakage around the needle. By infusing saline at relatively slow rates (e.g., 0.02 ml/min) clotting at the needle tip could be prevented up to 5 hr (the longest time tested). In deeply anesthetized animals an injection of 6 mg/kg succinyl choline (in a volume of about 0.1 ml) consistently produced termination of breathing in 2–3 sec. This indicates that the injection was IV, since the same dose given IP took a much longer time to take effect (about 5 min).

EXPERIMENT 1

Method

Animals. The animals were male albino Sprague-Dawley rats (Charles River Co.) weighing between 350–400 g.

Surgery and Apparatus. Rats were anesthetized with chloral hydrate (400 mg/kg, IP) and placed in a stereotaxic instrument. The skull was exposed by a midline incision and the skin and fascia retracted. Four 1/8 in stainless steel screws (0–80) were placed into the skull to serve as anchors for later application of dental cement. A small hole (No. 57 drill) was drilled through the skull at lambda, taking care not to puncture the sinus. The hub of a disposable 19 ga hypodermic needle, which had previously been cut so that only 1 mm of its needle and 14 mm of plastic remained, was then lowered stereotaxically into the hole. A convenient way of holding the hub in the stereotaxic is to place it onto a small syringe and hold the syringe with a syringe holder adapter (e.g., Kopf Universal holder No. 1271). To prevent clotting at the tip of the hub from obstructing its opening, prior to implantation a short piece of 20 ga wire can be inserted through the hub, cutting one end to be flush with the 1 mm tip and tightly crimping the other end over the wall of the hub. After the hub had been positioned into the hole a small amount of bone wax was beaded around the junction of the hub and the skull, to prevent cement from leaking under the skull. The hub was then cemented in place with dental cement and the animal allowed to recover (1 day to 1 month).

After recovery from this initial operation, the rat was anesthetized with halothane by inhalation and put back into the stereotaxic. Halothane rather than ether or chloral hydrate was used because of its short duration [12]. The 20 ga wire used to plug the hub was removed. A 27 ga, 1/2 in. hypodermic needle (regular point), connected via PE 50 Luer end intramedic tubing through a 2-way valve to a syringe and infusion pump (Harvard Apparatus), was then lowered stereotaxically through the guide hub. Successful penetration of the sinus was assured when slight negative pressure on the syringe (previously filled with sterile saline) pulled up an unbroken column of blood into the PE 50 tubing. If the needle had not penetrated yet, no blood was seen. If the needle had gone too deep, through the sinus, or was off midline, to the side of the sinus, greater difficulty in pulling back on the syringe was encountered and the fluid observed was only faint red, often frothy or bubbly. When successful penetration was achieved, the withdrawn blood was returned to the sinus and the valve switched to allow automatic infusion of sterile saline at a flow rate of 0.05 ml/min. Finally, the 27 ga hypodermic needle was cemented to the 19 ga plastic guide hub with a quick setting epoxy (e.g., Barco Bond MB 165–1 min cure time). With some practice, the procedure takes about 10 min.

Immediately after the epoxy hardened, the infusion pump was turned off and the rat removed from the stereotaxic. The valve connecting the animal to the syringe and infusion pump was removed and the PE 50 tubing (about 30 cm long) connected to a feedthrough cannula slip ring (Lehigh Valley No. 192–84) (Fig. 2).

On the other side of the slip ring a 2-way valve was attached; 1 side going to the infusion pump and the other side going to a drug-filled syringe (Fig. 2). Once the animal had been connected to the slip ring the infusion pump was turned to a flow rate of 0.05 ml/min and the animal allowed to recover from the halothane (about 10–20 min).

To infuse a drug, the infusion pump was turned off and the valve turned to connect the animal to the syringe. The desired volume of drug was pushed through the syringe, the valve returned to the pump side and the pump turned back on. The system was designed so that the volume of fluid between the animal and the valve was 0.6 ml. At a flow rate of 0.2 ml/min, 3 min was required from the time the drug was pushed through the syringe until it was completely

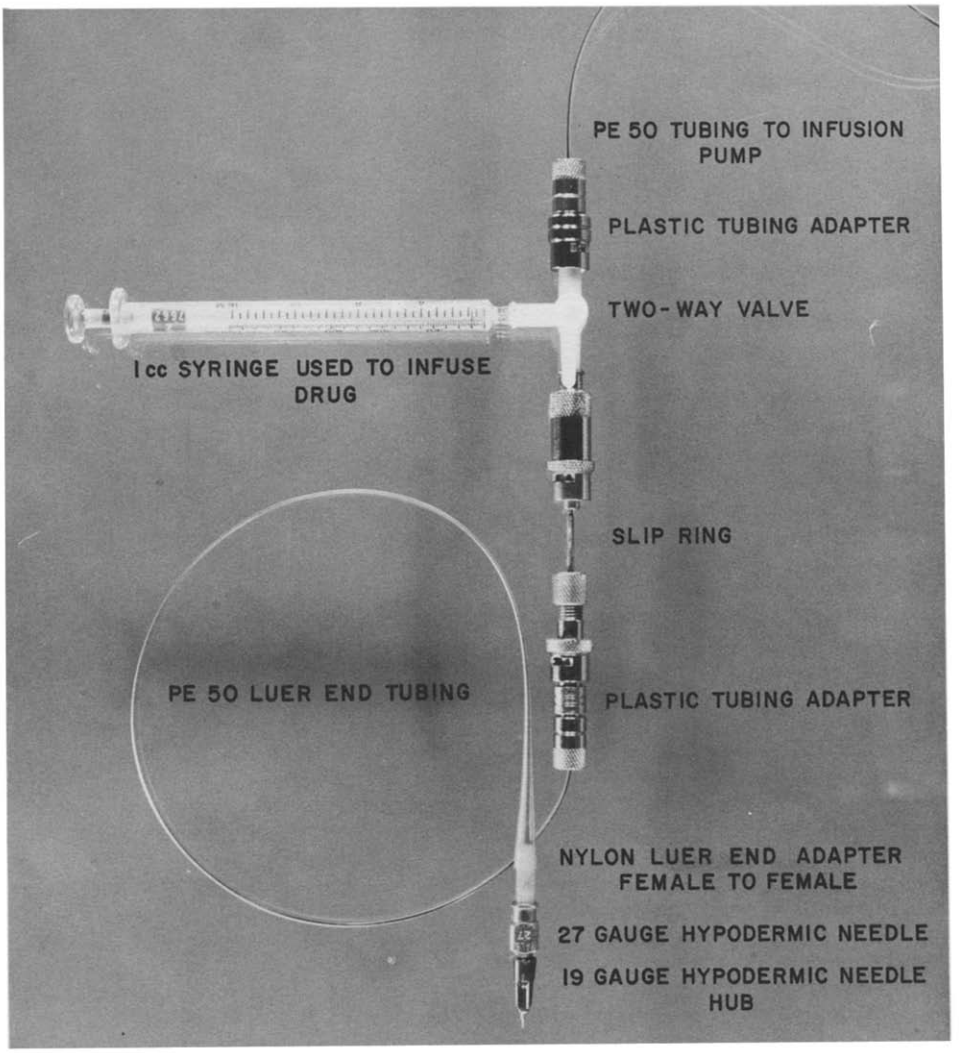

FIG. 2. Photograph of infusion apparatus. Note the tip of the 27 ga needle extending about 1 mm through the 19 ga hub. When implanted, the cut end of the 19 ga needle lies immediately above the sinus and the 1 mm point of the 27 ga needle lies inside the sinus. To prevent kinking of the PE 50 Luer end tubing when the animal moves around, the PE 50 tubing can be threaded through PE 320 tubing (not shown). One end of the PE 320 tubing is epoxied to the base of the plastic tubing adapter, the other end is fused with heat to the taper of the Luer and PE 50 tubing. In this way, it is still possible to have a small volume of fluid between the infusion syringe and the animal, but the connection between the rat and the slip ring is much stronger.

injected into the sinus. Slower or faster infusion rates can of course be used by varying the settings on the infusion pump. Once the drug had been injected into the sinus, the flow rate of saline was reduced to 0.05 ml/min to maintain patency for later drug injections.

Much of the apparatus used to measure startle has been described in detail elsewhere [6]. Briefly, a cylindrical wire and wooden cage (20 cm in dia., 20 cm high), suspended between compression springs within a steel frame, was used to measure startle. Cage movement resulted in displacement of an accelerometer, the output of which was proportionate to the velocity of movement. The amplified accelerometer output was fed directly into a Beckman dynograph. Startle amplitude was defined as mm of pen deflection. A 110 db, 50 msec white noise burst having a rise-decay time of 5 msec was used to elicit startle. Background noise was maintained at 70 db.

Procedure. Prior to implantation, 10 rats were presented with 10 noise bursts at a 20 sec interstimulus interval (ISI). Based on their mean startle amplitude across these 10 tones, the rats were divided into 2 groups of 5 rats each. One day later the rats were implanted with the guide hub as described above. One to 2 days later the infusion needle was secured in the sinus, as described above. After recovery from halothane (10–20 min) the rats were placed in the startle cage and 15 min later presented with startle-eliciting noise bursts at a 20 sec ISI. Five min later, rats from one of the matched groups were injected IV with saline (0.2 ml) and rats from the other group with LSD (15 μg/kg – free base in 0.2 ml). Subsequent injections were given 30 and 60

min later to test the reproducibility of the LSD effect. The time points were chosen on the basis of exploratory work which showed the effect of this IV dose of LSD lasted about 25 min. To determine if the needle was still in the sinus, immediately after testing all rats were injected IV with chloral hydrate (150 mg/kg). When deep anesthesia had developed, 0.2 ml of succinyl choline (6 mg/kg) were given IV, and the time required for cessation of breathing, which was easier to time accurately than the loss of the righting reflex caused by chloral hydrate, was recorded.

Results

Figure 3 shows the mean amplitude of startle over blocks of 10 tones after injection (as indicated by arrows) of either LSD or saline. Injection of saline had no detectable effect on either startle or the general ongoing activity of the animal. LSD, on the other hand, caused a rapid increase in startle amplitude which lasted about 25 min. Subsequent LSD injections again increased startle with a similar time course. An overall analysis of variance found a significant drug effect $F(1,8) = 11.34$, $p<0.01$) and a significant Drug × Time interaction $F(30,240) = 3.12$, $p<0.01$).

After being anesthetized with chloral hydrate, succinyl choline stopped every rat from breathing within 2–4 sec after infusion. In other experiments, clonidine (80 μg/kg) significantly depressed startle amplitude. This is consistent with other data which used the IP route [9], and indicates that depressant as well as excitant effects can be produced by this method of drug administration.

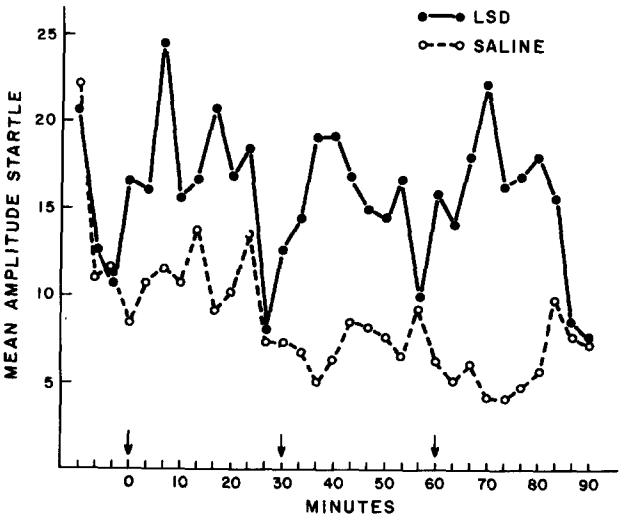

FIG. 3. Mean amplitude startle response over blocks of 10 tones after successive injections (arrows) of LSD (solid) or saline (dotted).

DISCUSSION

The results indicate that the junction between the transverse and sagittal sinuses is a feasible site for IV injection of drugs in freely moving rats. The technique involves relatively easy surgery and subsequent restraint of movement is minimal. For those already familiar with stereotaxic techniques, the method may be preferable to procedures which involve catheterization of the jugular or tail vein. At present, however, the technique has not been developed for long-term chronic infusion studies over many days. This would entail some way of firmly attaching the infusion needle to the guide tube during testing but also allowing its removal during intervals between testing, as well as determining whether multiple punctures of the sinus would be possible.

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