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# Automating the generation and collection of rate-frequency functions in a curve-shift brain stimulation reward paradigm

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**Abstract**

The electrical self-stimulation paradigm has proven to be very useful in research aimed at delineating the neural substrates involved in reward-related learning. Of the procedures currently employed the *curve-shift* method is among the more useful since it distinguishes between treatment effects on reward and performance. This method involves generating and plotting rate-frequency functions and quantifying the effects of experimental manipulations on reward by measuring the degree of lateral shift in these functions. We have designed a computerized system that automatically generates and collects descending rate-frequency functions from self-stimulating rats. The 3 main units of this system consisted of a 6809 micro-controller, a programmable timer logic board and a constant current source. The micro-controller and programmable timer operated on custom written software that monitored lever pressing in the operant chambers and controlled stimulation parameters to generate and record rate-frequency functions. The present report describes this system and presents some typical data collected from rats self-stimulating on ventral tegmental electrodes before and after the administration of intra-accumbens vehicle (0.5 $\mu$l distilled $H_2O$), (+)-amphetamine (20.0 $\mu$g/0.5 $\mu$l), quinpirole (10.0 $\mu$g/0.5 $\mu$l) and systemic quinpirole (1.0 mg/kg), all dopamine agonists. Stimulation consisting of 300-ms trains of cathodal rectangular pulses (0.1 ms) was available in 50-s trials. The number of pulses per train was decreased logarithmically from a value that sustained maximal responding to one that would not sustain responding. Self-stimulation thresholds were obtained by fitting the Gompertz growth model to the data and calculating the point of maximal acceleration of the sigmoidal curve. It was found that the present system generated and collected rate-frequency functions similar to those that have been obtained manually in previous experiments. The data showed that the system was sensitive to both central and systemic pharmacological manipulations by producing lateral and vertical shifts of the rate-frequency functions, indications of reward and motor effects, respectively. It was concluded that the present design was useful in conducting entire self-stimulation sessions that required minimal monitoring by the experimenter.

*Key words:* Automation; Curve-shift; Brain stimulation reward; EC board; ECBASIC; Operant responding; Method; Reinforcement; Reward; (Rat)

## 1. Introduction

In 1954 Olds and Milner discovered that rats would *self-administer* electrical stimulation to discrete areas of their brains when this stimulation was contingent on pressing a lever. This finding suggested that the electrical current might have been activating neuronal elements of the brain's motivational and reward systems. This discovery constituted one of the most important and influential discoveries in physiological psychology. Since then the self-stimulation paradigm has been used extensively in the search for the neuroanatomical and neurochemical substrates of the brain's reward systems (for detailed reviews see Wise and Rompré, 1989; Milner, 1991).

Earlier self-stimulation studies determined the rewarding efficacy of electrical stimulation through methods that relied exclusively upon the rats' rate of responding. For example, with the *constant-current* method, reward efficacy was determined by measuring the rate at which rats would press a lever to obtain a fixed amount of stimulation. With the *constant-re-*

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*sponse* method, reward efficacy was determined by measuring the amount of stimulation required to produce a pre-determined rate of responding. It has been shown that reward-irrelevant factors (e.g., motor impairment or sedation) can influence the rate at which rats press a lever for stimulation (Roll, 1970; Rolls et al. 1974; Miliaressis et al., 1986). This influence made it difficult to determine whether the effects of a manipulation (e.g., injection of a pharmacological compound) were on the reward efficacy of stimulation or on the rats' ability to perform the response. We concur with others (Edmonds and Gallistel, 1974; Wise and Rompré, 1989; Miliaressis et al., 1986; Stellar et al., 1988) that reward efficacy of stimulation is measured more adequately using rate-free methods.

One method that distinguishes between reward and performance variables was first described by Edmonds and Gallistel (1974). They proposed that a psychophysical method referred to as the *curve-shift* paradigm can enhance the resolution of reward measurement by minimizing the possible confound of changes in performance. Generally, the method involves administering trains of rectangular pulses and plotting a rate-frequency function that depicts the animals' level of responding ($Y$ axis) across a range of stimulation frequencies ($X$ axis). A typical rate-frequency curve resembles a sigmoidal function. Validation experiments demonstrated that shifts in the lateral position of the curve were a selective measurement of stimulation-produced reward whereas vertical shifts in the asymptote were indications of the animals' ability to perform the required response (Edmonds and Gallistel, 1974; Edmonds et al., 1974; Miliaressis et al., 1986; Stellar et al., 1988). The curve-shift method is especially useful when investigating the effects of pharmacological agents on self-stimulation.

We have designed and built a computerized system that automatically generates and collects rate-frequency functions from self-stimulating rats. This system collects a user-defined number of rate-frequency functions before and after (pharmacological) manipulations through the use of a micro-controller, custom designed programmable timer logic circuits, a constant current source and controller software.

Other investigators (Campbell et al., 1985; Kling-Petersen and Svensson, 1993) have described computerized methods that perform some of the functions reported here. However, unlike our method, theirs do not include the design and building of hardware but rely on the use of commercially obtained equipment (e.g., NB-TIO-10 (National Instruments, Austin, TX) interface card and physiological stimulators). Furthermore, our system can be used in auto-titration (2 levers) or 2-electrode experiments (both electrodes controlled by 1 stimulator), features that are lacking in other set-ups.

We tested the ability of our system to conduct entire self-stimulation sessions during which animals were tested with compounds known to have effects on operant responding for reward. The present report describes the system, including its electronic circuits, and presents results demonstrating the system's effectiveness.

## 2. Materials and Methods

Treatment of rats in the present study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policy and was approved by the Queen's University Animal Care Committee.

### 2.1. Subjects

Twenty-five male Wistar rats (Charles River, Canada) weighing between 275 and 325 g at the time of surgery ($\approx$ 5–7 days after arrival) were housed in individual hanging cages and maintained on a 12-h light/dark cycle (lights on at 07:00 h) in a temperature-controlled environment (21°C). Purina rat chow and water were available to the rats ad libitum.

### 2.2. Surgery

The rats were anaesthetized with sodium pentobarbital (Somnotol) at 65 mg/kg of body weight and fitted into the stereotaxic apparatus. Moveable electrodes (Miliaressis et al., 1982) were implanted in the ventral tegmental area (VTA) using the stereotaxic coordinates of 4.8 mm posterior to bregma, 2.5 mm lateral from the midline with the electrode at an angle of 10° off vertical and moving medially and 8.5 mm below the surface of the skull. Cannula guides (diameter: 0.64 mm) were aimed at the nucleus accumbens using the coordinates of 2.2 mm anterior to bregma, 1.5 mm lateral from the midline and 7.0 mm below the surface of the skull. The incisor bar was positioned at 3.5 mm below the horizontal plane passing through the interaural line (Paxinos and Watson, 1982).

Electrodes consisted of a plastic guide and a moveable stainless steel wire (diameter: 0.25 mm) coated with epoxylite, except for the tip which was honed to a hemispherical shape. The cannula guides were made from modified stainless steel needles (diameter: 0.64 mm) cut to a length of 14 mm. The injector guides were made from stainless steel tubing (diameter: 0.32 mm) to achieve a length of 15 mm. A stainless steel wire (diameter: 0.32 mm) was kept in the guide cannula between injections.

### 2.3. Apparatus

#### 2.3.1. General description of programmable pulse-pair stimulator

The programmable pulse-pair stimulator consisted of an Apple Macintosh (MACPlus) host computer (see Weisman and Palya, 1988 for details on how to set up the MAC), a 6809 based micro-controller (Experiment Controller (EC) board) (Walter and Palya, 1984), a programmable timer logic board and a constant-current source. The MACPlus computer was used to upload custom written software for running experiments to the EC board and to download data to be analyzed by the experimenter. Red Ryder 10.3, a terminal communication software package, was used to transfer the information.

The EC board was programmed (with ECBASIC-similar to BASIC but with commands suited to running experiments) to monitor lever press responses and to change stimulator parameters during the course of a session. The programmable timer logic board was interfaced to the EC board via the controller's data and address bus in the same way that memory is interfaced to a computer. The EC board selected timers and wrote data to registers signifying pulse parameters. When a pulse train was desired the software enabled a start signal to the timer logic board. The timer logic board then delivered a pulse train, with parameters previously programmed by the EC board, to the constant-current source. These pulses switched on and off the constant-current source connected to the animal. Bar presses were fed back to the EC board where they were analyzed immediately to determine the next steps in the procedure. This software automatically produced rate-frequency functions.

This type of configuration permitted the simultaneous running of many animals with minimal need for monitoring by the experimenter. Thus, the previously manual tasks of systematically adjusting switches and potentiometers to produce stimulation pulses, of moni-

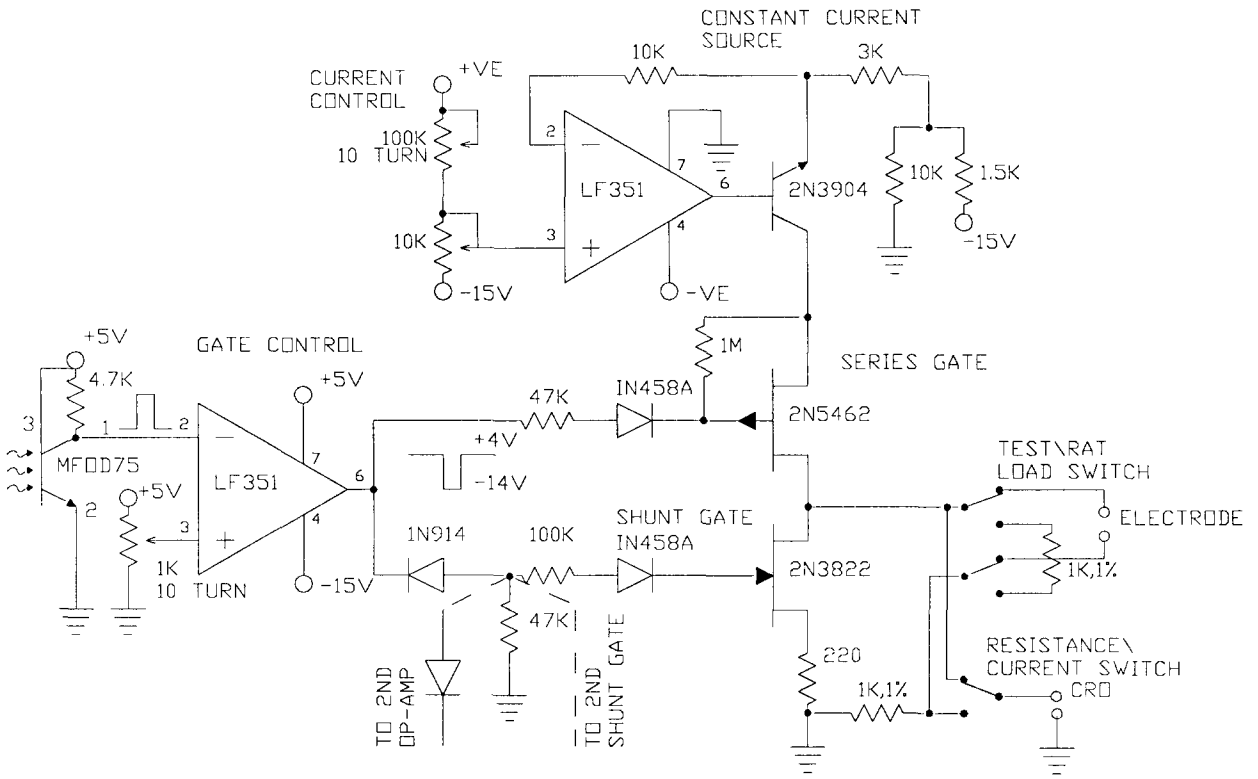


Fig. 1. Schematic diagram of the *constant-current source*. The two potentiometers at the top left control the current level. The 100 k$\Omega$ potentiometer was used to zero out the current when the main potentiometer was at 0 $\Omega$. As the voltage on pin 3 of the LF351 changes, the output voltage changes the biasing of the 2N3904 in an attempt to keep the voltage at pin 2 and 3 the same. This in turn creates a constant current source. The gate control circuit will switch on the series gate (2N5462) during a pulse and switch off the shunt gate (2N3822). The TEST\RAT switch determines which load will receive the current. The test load is a 1 k$\Omega$ resistor which is selected while the initial current level is adjusted. The RESISTANCE\CURRENT switch determines from which side of the load voltage measurements will be made. If voltage is measured across the 1 k$\Omega$ resistor then current can easily be calculated. 1 mV represents 1 $\mu$A of current. If voltage is measured across the rat then rat resistance can be calculated. The broken lines in the diagram show how 2 stimulatiors are to be interconnected so that the shunt gates can function properly; both shunt gates must be 'off' whenever a stimulation pulse is present on either of the 2 electrodes (see Mundl, 1982). The 2-electrode set-up was not used in our study. The connection between the gate control and the timer logic board was via a fiber-optic cable. This method was used to decrease noise picked up by the constant current source.

toring the end of one rate-frequency function and beginning of a new one and of recording the number of lever presses for every trial of an entire experiment have been fully delegated to computers.

### 2.3.2. Description of individual components

*Current stimulator*. Current intensity was adjusted with a 10-turn potentiometer that controlled the input voltage of an operational amplifier (see Fig. 1). As this input voltage changed from −13 V to −4.4 V, the current intensity changed linearly from 0 μA to −2.5 mA. The constant-current source is similar to the one described by Mundl (1980) with the exception of a modification to the biasing of the gate circuitry, viz., the replacement of transistors with a single operational amplifier.

A programmable timer logic circuit (described below) switched on and off the constant-current source via a fiber-optic link. The fiber-optic link allowed complete electrical isolation between the constant current source and the control part of the system. Furthermore, it allowed us to place the control circuit out of the experimental room, reducing the effect of electromagnetic and radio frequency interference. The optical signal was detected with an optical sensor connected to an operational amplifier, configured as a voltage comparator. This comparator transformed a logic pulse of 0 to +5 V into a pulse of −12 to +5 V, capable of biasing the series and shunt gates of the current stimulator. The shunt gate prevented the build up of electrical charge on the electrode/tissue interface as it conducted between pulses and was non-conducting during the stimulation pulse. The series gate, conducting current during pulses and non-conducting between pulses, isolated the electrode from the constant-current source, preventing cross-talk in 2-electrode systems (used in studies aimed at determining the direction or velocity of neuronal impulse flow (see Shizgal, 1989)). Fig. 1 includes the connection of 2 stimulators for use in a 2-electrode system. The function of these gates is described in more detail in Mundl (1980).

Current delivered and/or animal resistance was monitored with an oscilloscope. The oscilloscope leads

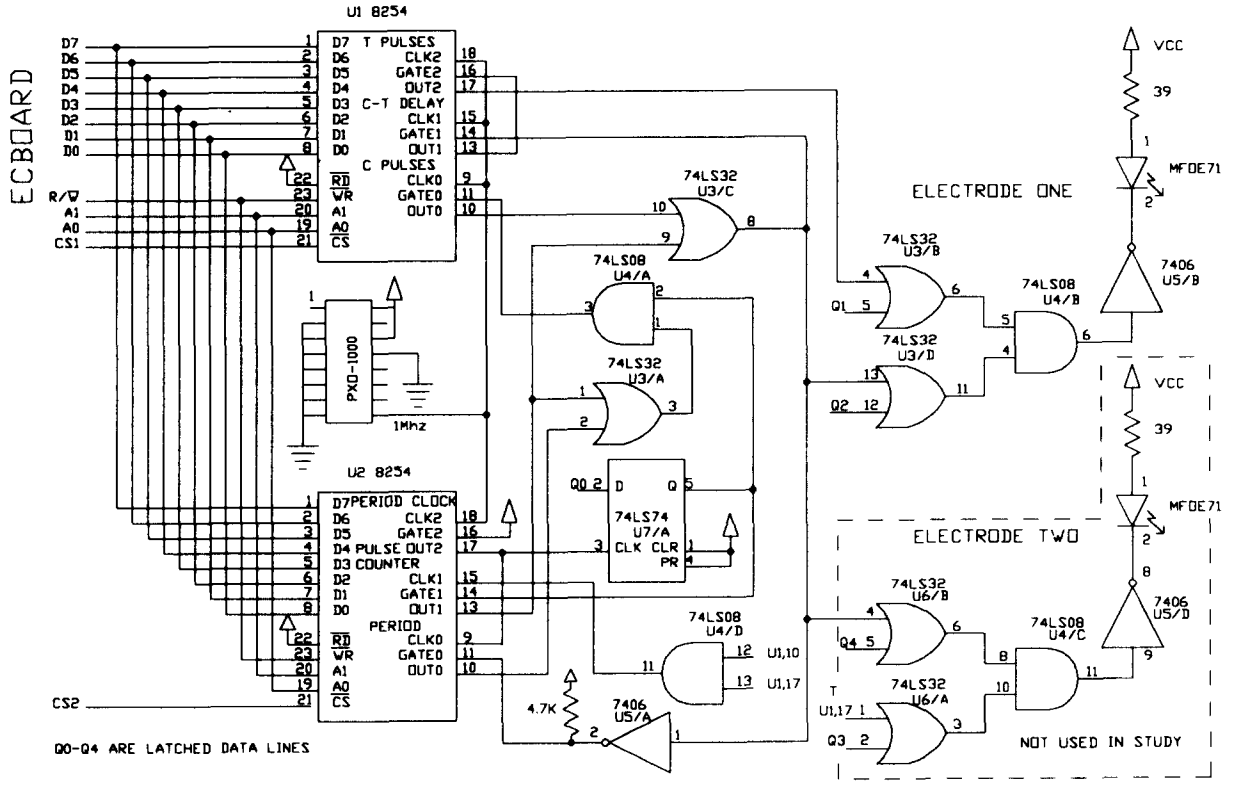


Fig. 2. Schematic diagram of the *programmable timer logic board*, initially designed for a 2-electrode system administering C-T pulses, as shown here. We utilized only the C function of the circuit and 1 electrode, but initial tests on both functions showed that the entire circuit worked perfectly. The timer integrated circuits (ICs) consist of the Intel 8254 and are clocked with a 1 MHz signal. They are interfaced to the micro-controller and are completely controlled by software. The other ICs located on the board are simple TTL ICs. Latched data lines Q0-Q4 are used to enable pulses to the 2 electrodes. Q0 and Q1 determine whether T and C pulses, respectively, are delivered to electrode 1. Similarly for Q3 and Q4 and electrode 2. Q0 going high is used to initiate a pulse train. The pulse train ends when the desired number of pulses is counted with the pulse counter (U2,1) which in turn outputs a low, disabling the gate of the C pulse timer. The interface between the programmable timer logic board and the constant-current source is via a fiber-optic connection which is driven by MFOE71, an infrared LED.

were switched with a SPDT switch in order to measure the voltage potential across a 1 kΩ resistor or to measure the voltage potential across the animal and the 1 kΩ resistor in series. Current delivered was calculated as the potential voltage measured across the 1 kΩ resistor divided by the resistor value. For example, to achieve a pulse current of 500 μA, the potentiometer was adjusted until a voltage of $V = (I \times R) = (500\ \mu A \times 1K) = 500$ mV was measured on the oscilloscope. The resistance of the rat was calculated by measuring the voltage potential difference across the rat and dividing this by the known current. For example, if the voltage measured across the rat was 5 V and the current delivered was 500 μA then the resistance would be $R = V/I = (5/500\ \mu A) = 10$ kΩ. This information was useful for the positioning of electrodes.

The output of the stimulator was sent to a DPDT switch, allowing the experimenter to select a test load while initially adjusting current intensity. In our configuration, software was written that would switch on the stimulator by pressing a TEST button, thereby allowing the user to set the desired current intensity. After the desired level was reached the DPDT switch would be returned to the correct position and the TEST button would be pressed again to end the test pulses. This method of adjusting current eliminated the need for a counter dial on the potentiometer.

*Programmable timer logic circuit.* Pulse parameters were controlled by two programmable timer/counter integrated circuits (U1,U2) interfaced to the EC board (see Fig. 2). The integrated circuits were connected directly to the data bus of the EC board and were addressed with extra address locations available on the EC board. Other signals from the EC board were data lines, A0, A1, R/W and 5 latched output lines for enabling pulse signals on the timer logic circuit. The 2 timer chips were programmed to control pulse duration, pulse frequency and the number of pulses per train (automatically or manually by pressing the INC or DEC buttons which caused the software to reprogram the programmable timers to the desired number of pulses, see Fig. 3).

The timer/counter chips were programmed as follows. The timer labeled C pulses (U1,0) was programmed as a programmable 1-shot. When the user enabled a pulse train by setting Q0 high, a low-to-high transition occurred on the gate of the C pulse timer.

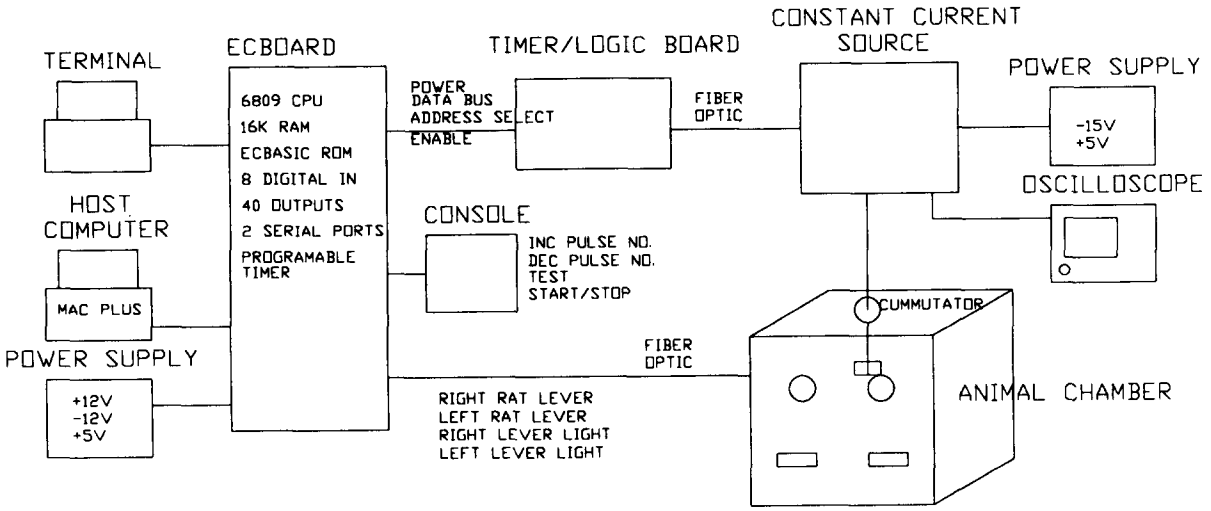


Fig. 3. Block diagram showing the main components of the system. The entire system centers around the EC board. In a sense, it becomes the experimenter during a session. Instead of a person manually adjusting pulse parameters and monitoring and recording lever presses, the EC board automatically does it. Blocks shown in the diagram are as follows. The terminal was connected to a second serial port on the EC board which was used to output session messages to the experimenter. Messages included the threshold and trial number, the number of pulses available, the number of bar presses, and whether a session was over. This information gave the experimenter an immediate indication of how the rat was performing. The host computer was connected to the main serial port of the EC board and was used only for the transfer of software before a session and the transfer of data afterwards. The host computer was disconnected from the experiment while a session was in progress, giving other people in the lab access to the computer. The EC board was powered by an old personal computer power supply delivering +5 V, +12 V and −12 V. The programmable timer logic board was interfaced to the EC board via a ribbon cable connection and was housed in the same enclosure as the EC board. The enclosure included a console with button switches enabling the experimenter to manually control test pulses, number of pulses and start of session. The constant current source was connected to the programmable timer logic board via a fiber-optic cable. The current source was housed in an enclosure with a control console. The console consisted of a current adjusting potentiometer, a switch to select between a test load and a rat, a switch to select which side of the load to take voltage measurements and a connector for the oscilloscope. The current source received its power of +5 V and −15 V from a dual power supply. The electrode leads went to a commutator which allowed the rat to turn freely within the operant chamber. The chamber had 2 houselights and 2 levers. The lights were controlled by the EC board via a fiber-optic connection and the levers were read by the EC board via a fiber-optic connection. The fiber-optic cables ran between 2 rooms, separating the control and current sections of the system.

This caused the C timer output to go low for the duration of the C pulse count. The count lasted up to 65,535 μs with a resolution of 1 μs. The C pulse going high triggered the C period timer (U2,0). The period timer was configured as a programmable 1-shot with an adjustable clock. The clock was generated from the period clock timer (U2,2) programmed as a rate generator. This allowed for a C period ranging from 2 μs to 1.2 h. The number of pulses delivered were counted with a timer (U2,1). The output of the counter was ORed with the output of the C period timer. The C period timer, in turn, would trigger the next C pulse, until the desired number of pulses had been delivered. At this point, the output of this counter disabled the C pulse gate thereby disabling the entire pulse circuit. The output remained in this state until the next pulse train was triggered by the EC board, setting Q0 low and then high again.

If pulse parameters needed to be changed, the programmable timers would be reprogrammed during the time between pulse trains. Parameters which would be changed during a session were number of pulses, period between pulses and possibly the period clock. The EC board would calculate the necessary changes to the timers and reprogram them with the new parameters.

The circuit diagram shows the connection for T pulses. T pulses were not used in the experiment and hence will not be explained in detail. T pulses were simply the product of a programmable 1-shot and a timer that controlled the C-T delay. It is recommended that if the circuit were to be replicated, the T hookup should be included. Software would then determine whether T pulses were delivered.

*Micro-controller*. The EC board consisted of a 6809-based micro-controller with 8 logic input lines, 40 available output lines, a RS232 serial port, 16 Kb of RAM and ran a modified BASIC language known as ECBASIC (see Fig. 3). It also had a built-in timer which allowed the experimenter to write time-dependent code. The EC board possessed additional address locations used to select the programmable timer logic chips. The EC board was powered by a +5 V, +12 V and −12 V supply, also used to power the timer logic board. As well as being interfaced to the timer logic board, the EC board controlled an 'in session' indicator (LED) and 2 cue lights, one above each lever. The EC board monitored 6 input lines which included Start/Stop, Test, increase (INC) pulse no., decrease (DEC) pulse no. and depression of the 2 levers.

The Start/Stop input was used to start and abort a session. After uploading software to the EC board via a serial connection from the host computer, the experimenter answered software prompts that determined the parameters of the session. These parameters include the rat ID, choice of single- or double-electrode session, whether or not the session was a drug test (drug testing sessions contained an indefinite pause between the end of the baseline determinations and the beginning of drug test deterinations to allow time to administer the drug), number of threshold determinations during baseline testing and length of post-drug testing after administration of drug, number of pulses per train to begin the session, the width of a pulse train, the width of a pulse, the inter-train interval, length of a trial, the inter-trial interval and the number of priming trains. Rarely would these parameters require changing throughout the course of a rat's participation in an experiment. Thus, these parameters were typically written to a separate text file for each rat and these files were transferred to the EC board and read by the ECBASIC program.

After the session parameters were set the experimenter used the Test input to manually adjust the current intensity for each rat and then pressed the *start* buttons to begin the sessions. Pressing the *start* button again aborted the session. A normal session would end after the desired number of thresholds had been reached or the desired amount of post-drug time had expired.

Lever presses served as inputs to the EC board and were monitored in the following manner. Lever presses were scanned with an assembly language incorporated into ECBASIC. The EC board assigns a variable to lever presses (closure of the lever switch for more than 30 ms) and increments this variable by a value of 1 with every response. The lever press variable was monitored throughout a trial (including during the delivery of a stimulation train). At the end of each trial the number of lever presses would be stored as data. Thus, lever presses, not stimulation trains, were accumulated for each trial.

Data was collected and stored in a text file. The user had the option of storing the data in one or both of two formats; one format was ready for spreadsheet manipulation and the other was ready to be analysed by a curve-fitting program. This program, based on the Gompertz sigmoid model, plotted a curve of the data and calculated the threshold (defined as the point of maximal acceleration on the rising portion of the curve) as well as the slope and asymptote of the curve (see Miliaressis and Coulombe, 1987).

To conduct an automated self-stimulation session the experimenter could execute the custom written software that automatically monitored the experiment, collected the data and programmed the stimulator using experimenter-defined criteria.

### 2.3.3. Operant chambers

The experimental environments consisted of four similar operant chambers (29 × 23 × 18 cm high) constructed of aluminum sides and plexiglass backs, tops

and doors. The top of each chamber contained a hole 4 cm in diameter to allow the stimulation lead to pass into the chamber. The floors were made of aluminum grids. Each chamber was placed in a ventilated sound-attenuating box. One of the 29 cm walls contained two 3.5 × 2.0 cm levers 8 cm apart. A force of 0.09 N was required to depress each lever. Only one lever was connected to the stimulator and lever press counter. A 2-W light bulb was situated 10 cm above each lever. The onset and offset of the lights signified the start and end of a trial (50 s), respectively.

### 2.3.4. Procedure

Following more than 1 week of postoperative recovery, the rats were tested for self-stimulation using 0.3-s trains of cathodal rectangular pulses each lasting 0.1 ms in duration. During the shaping period the current intensity and frequency were varied manually and finally fixed at parameters that maintained bar pressing. The rats then were allowed to press the lever freely for 1 h daily for 4 consecutive days. Self-stimulation thresholds then were determined by setting the frequency of pulses at the value that induced maximal responding and decreasing the frequency by decrements of approximately 0.055 log units of pulses until the rats stopped responding. Stimulation was available to the animals for trials of 50 s with an inter-trial interval of 15 s. Presses on the rewarded lever were counted.

The testing period began when the self-stimulation threshold was stable for each subject. A stable threshold was operationally defined as 3 consecutive sessions during which the threshold (calculated using the Gompertz sigmoidal model, see Coulombe and Miliaressis, 1987) did not vary by more than 10%.

The testing period consisted of 4 test sessions each separated by at least 48 h. Each rat was tested with intra-accumbens injections of distilled water vehicle (0.5 μl), (+)-amphetamine (20 μg/0.5 μl), quinpirole (10 μg/0.5 μl) and systemic quinpirole (1.0 mg/kg, i.p.) in a counterbalanced order. Test sessions began with 4 new determinations of self-stimulation threshold after which the rat was removed from the operant chamber and administered vehicle or one of the drugs. In all conditions the rats were returned to the operant chamber and new determinations of self-stimulation threshold were obtained for 60 min.

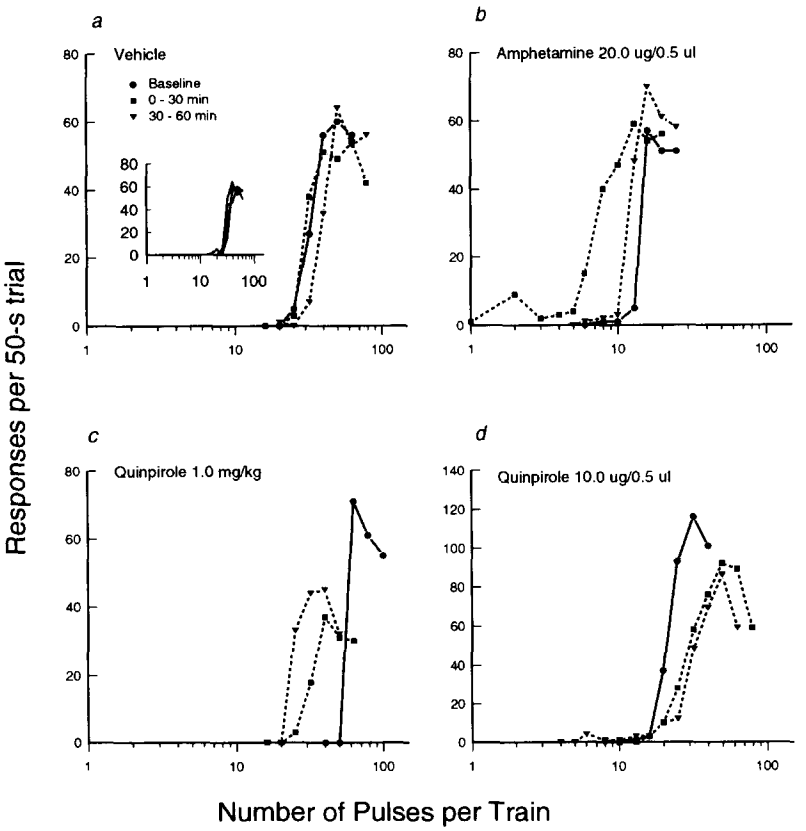


Fig. 4. The number of presses on the rewarded lever as a function of the number of pulses per train in each 50-s trial during which stimulation was available. Rate-frequency functions were determined by logarithmically decreasing the frequency of pulses from a value that induced maximal responding to one that induced no responding (thresholds). Each graph (except the insert in 4a) depicts 1 rate-frequency function from the pre-treatment period and 1 from each 30-min segment of the post-treatment period. The insert in 4a shows the 4 rate-frequency functions obtained during the pre-treatment period.

For central injections the rats were fitted with an injection cannula connected to a 10-$\mu$l Hamilton microsyringe through a length of polyethylene tubing. Using an infusion pump (Sage Instruments) injections (0.5 $\mu$l) were delivered over a 30-s period. The injector was left in place for an additional 60 s to ensure diffusion of the drug. (+)-Amphetamine (Smith, Kline and French, Canada) and quinpirole (Eli Lilly) were dissolved in distilled water.

## 3. Results

Fig. 4 depicts representative rate-frequency curves for each treatment condition from different rats. Each graph contains 1 rate-frequency curve obtained just before and 1 from each 30-min period after drug administration. In general, the data demonstrate that systematically decreasing the number of pulses in a stimulation train resulted in decreased responding for the stimulation. When responding across the range of frequency values was plotted using a log scale for the *X* axis the rate-frequency functions acquired a sigmoidal shape consisting of 3 distinct segments: a sub-threshold, a dynamic and an asymptotic segment. In the sub-threshold segment the rate of responding did not appear to increase as the number of pulses in a stimulation train increased. However, increasing the number of pulses per train eventually produced high rates of responding. The point at which this occurred is referred to as the *threshold* or *locus of rise* and signified the end of the sub-threshold portion and the beginning of the dynamic range of the rate-frequency curve. Within the dynamic range of the curves responding increased at a very steep rate with increases in frequency. The dynamic range ended at a point where further increases in the number of pulses per train did not result in relative increases in responding; in this case the animals were responding at their maximal or asymptotic levels. The pattern of responding depicted in Fig. 4 was typical of all rate-frequency functions obtained using the present equipment.

Fig. 4a shows the pre-treatment and 2 consecutive post-treatment rate-frequency functions for a representative rat that received an intra-accumbens injection of vehicle (0.5 $\mu$l of distilled water). As might have been expected, the lateral position and asymptote of the post-treatment function obtained during the first 30 min after treatment was very similar to the pre-treatment function. The second function (30–60 min) showed a small shift to the right of the pre-treatment function. This finding was typical of all rats that received a vehicle injection or no-injection. When thresholds were averaged across several animals it was found that the post-treatment thresholds in the second 30-min period were approximately 5% greater than pre-treatment values.

Fig. 4b shows the results obtained from a rat given an intra-accumbens injection of 20.0 $\mu$g/0.5 $\mu$l of (+)-amphetamine. The rate-frequency curve obtained during the first 30 min after administration of (+)-amphetamine was clearly shifted to the left of the pre-treatment curve. The data demonstrate that frequencies that previously did not support responding did so at asymptotic levels after the administration of intra-accumbens (+)-amphetamine. This effect appeared to have been short lasting since the 30–60 min rate-frequency function was shifted back towards the right and almost back to the pre-treatment position.

Fig. 4c shows the results of systemic injections of quinpirole. Like amphetamine, quinpirole shifted the rate-frequency curve to the left of the pre-treatment curve. However, the effects of systemic quinpirole also lowered the asymptote compared to the pre-treatment condition. Interestingly, when quinpirole was microinjected directly into the nucleus accumbens (Fig. 4d) the result was a rate-frequency curve shifted to the right of the pre-treatment curve. These results were typical of all rats receiving these treatments.

## 4. Discussion

The present results demonstrate that the rates at which rats will respond for electrical stimulation of the brain can be influenced by the number of pulses available in the stimulation trains. Thus, the greater the number of pulses in a train the greater the rate of responding for the stimulation. This was especially true for the frequencies within the dynamic range of the rate-frequency functions. It appears that the self-stimulation system possesses the ability to add the rewarding effects of several pulses arriving over a short period of time, a phenomenon referred to as *temporal summation* (see Milner, 1991). The results also demonstrate that the sum of the rewarding effects of these pulses must exceed a threshold level before the stimulation is effective as a reward. Finally, the data show that the self-stimulation rates will reach an asymptotic level where further increases in frequency do not produce higher responding. Perhaps this indicates a limitation in responding due to reward-irrelevant constraints on performance resulting from stimulation of any particular site. This interpretation is supported by findings which show that although rats may respond at the same rate for two different sets of stimulation parameters they will consistently choose the higher of the two (Miliaressis and Malette, 1987; Waraczynski et al., 1987) and work harder to gain access to the higher stimulation parameters (Hawkins and Pliskoff, 1964) (see Wise and Rompré, 1989; Stellar et al., 1988)

The present data show that when rates of responding for a range of frequencies of electrical pulses to the brain are plotted the result is a sigmoidal function. The present data are in agreement with several previous studies that have manipulated similar stimulation parameters manually (Miliaressis et al., 1982; Rompré and Miliaressis, 1985; Gratton and Wise, 1985; Miliaressis et al., 1986). Unlike other reports of automated systems (Campbell et al., 1985; Kling-Petersen and Svensson, 1993) we have not included any statistical analyses of our results. The purpose of this experiment was to attempt to automatically generate characteristic rate-frequency functions. Statistical analyses have no bearing on this. Furthermore, because of a lack of convergence of opinion on which are the best indices of threshold we decided to leave this matter completely aside. Thus, an advantage of the present system over other automated ones is that the user is not bound to particular types of statistical analyses.

The present data show that the rate-frequency functions obtained after an injection of vehicle (and no-injection for which data were not shown) are shifted slightly to the right of those obtained before the injection. These data suggest that the post-treatment self-stimulation threshold is slightly higher than the pre-treatment value. These findings are in accord with some previous studies that also collected descending rate-frequency functions from 50-s trials with 15-s inter-trial intervals as used here (Rompré and Wise, 1989a,b). These data suggest that the self-stimulation system becomes less sensitive to stimulation with continued use, resulting in slightly elevated thresholds. This interpretation is supported by the finding that thresholds were higher in the second 30-min period than in the first 30-min period after vehicle administration.

Intra-accumbens injections of (+)-amphetamine shifted the rate-frequency function to the left, an effect that is interpreted as an enhancement of the rewarding efficacy of VTA stimulation. Intra-accumbens (Colle and Wise, 1988) or systemic (Gallistel and Karras, 1984) (+)-amphetamine previously have been demonstrated to produce leftward shifts of the rate-frequency function in other curve-shift experiments. Other investigators using automated curve-shift set-ups (Campbell et al., 1985; Kling-Petersen and Svensson, 1993) also have reported an amphetamine-produced shift to the left of the rate-frequency function.

Systemic administration of quinpirole shifted the rate-frequency function to the left, a finding in accord with the report of Nakajima and O'Regan (1991) that quinpirole induced an enhancement of the rewarding efficacy of lateral hypothalamic stimulation. However, intra-accumbens injections of quinpirole produced shifts of the rate-frequency function to the right, suggesting that it impaired the rewarding efficacy of VTA stimulation. The differential effects of systemic and intra-accumbens quinpirole suggest that the reward-enhancing effects of systemic quinpirole likely do not occur in the nucleus accumbens. Other studies in our laboratory suggest that these effects likely do not occur in the caudate-putamen or cortex either (Ranaldi and Beninger, 1993). Further studies are needed to explore the location of the quinpirole enhancement of rewarding stimulation.

The present computerized system for generating and collecting rate-frequency functions appears to have advantages over manual and automated systems. With the entire experiment being automated, there is no chance of experimenter error in adjusting stimulation parameters. The controller calculates all session and stimulation parameters during an experiment and modifies these parameters to create a rate-frequency function. Data analysis can, similar to Kling-Petersen and Svensson's (1993) method, be greatly facilitated through automation (with any of several commercially available, easy-to-use data management software packages). Since the programming is in a higher level language (EC-BASIC) it is possible to make changes to the procedure to suit different requirements (e.g., C-T pulses or multiple lever presses required for reward, etc.). Furthermore, additional systems can be built so that more than 1 rat can be tested at a time. In our laboratory we have built 4 systems that allowed for 4 sessions simultaneously. In this case the EC boards were networked to the host computer, with each EC board being given an address (see Walter and Palya, 1984). One advantage of this system over that of Campbell et al. (1985) is the present one's ability to change pulse and train duration settings *during* a session. Because in their set-up these settings are set manually through rotary switches this function cannot be performed. Another advantage of the present system is that it counts *all* lever presses and not just those which result in stimulus train delivery as is done in the set-up of Campbell et al. (1985). Thus our system provides a more accurate account of performance output which is directly related to the motivation to self-stimulate and to the motor effects of pharmacological manipulations. In addition, our set-up accomodates the use of 2 electrodes permitting experiments aimed at investigating connectivity, directionality and conduction velocity of impulse flow. Finally, another advantage of our system over other presently available automated systems is that the present one was designed to be easily constructed from electronic parts that are cheap, readily available and easily obtained through electronic suppliers. Because each of the present systems can be built for very cheap laboratories can increase the number of sessions running simultaneously and, thus, enhance the rate of data collection.

In general, the automated system used in the pre-

sent study generated and collected rate-frequency functions that were comparable to those obtained manually and with other automated systems. These automatically collected rate-frequency functions were useful in determining the effects of drugs on brain stimulation reward.

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