Contingent and Non-Contingent Intracranial Electrical Stimulation Using the Raturn™

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We describe the use of the Raturn™ for investigation of behavior associated with contingent and non-contingent (or experimenter applied) intracranial electrical stimulation in the rat. The results support previous qualitative observations by our laboratory and may yield insight into the role of the neurotransmitter dopamine in the brain reward system.

The brain reward system (BRS) mediates naturally reinforcing stimuli such as food, water, and sex, and it is also the target site for drugs of abuse (1). Considerable insight into this important neural circuitry has been provided by the technique of intracranial electrical stimulation. For example, experimental animals can be trained to press a lever in order to obtain a reinforcing train of pulses applied to an implanted stimulating electrode. This interesting behavior, called intracranial self-stimulation (ICS), was first observed by Olds and Milner (1954) over four decades ago (2). Since that time, ICS has been extensively used to identify the anatomy (3), neuronal characteristics (4) and pharmacology (5) of the BRS. Non-contingent (or experimenter-applied) intracranial electrical stimulation has also been employed to study similar phenomena (6-8).

We recently observed that brain levels of the neurotransmitter dopamine, measured in freely behaving rats by real-time microsensors (9), were increased by experimentally-applied electrical stimulation but were unchanged during ICS, despite the use of identical stimulation parameters (10). This result is consistent with a function of dopamine other than the long held view of a neural substrate for reward (11-13). Evoked behavior also appeared to be distinct for the type of intracranial stimulation as non-contingent stimulation, in particular, elicited a profound behavioral activation. Unfortunately, differences in the behavioral responses to electrical stimulation were only described anecdotally.

In this study, we evaluated the Raturn™ from Bioanalytical Systems, Inc. to assess behavior quantitatively during contingent and non-contingent electrical stimulation. The Raturn is a swivel-free containment system for combined neurochemical and behavioral monitoring (covered by US Patent No. 5,816,256 and European Patent Application No. 0872179). The existing system was shown to assess behavior elicited by experimentor-applied stimulation successfully without modification. However, it was necessary to adapt the Raturn to perform ICS by incorporating a lever press. Two designs were tested. The first design utilized a commercially available omnidirectional lever (Lafayette Instruments), which could be lowered vertically into the containment bowl. For the second design, a "platform" press was specially constructed to fit into the base of the containment bowl and be activated when the animal stepped on it. Although both designs supported ICS, the platform press appeared to elicit additional behaviors not previously associated with ICS.

Materials and Methods

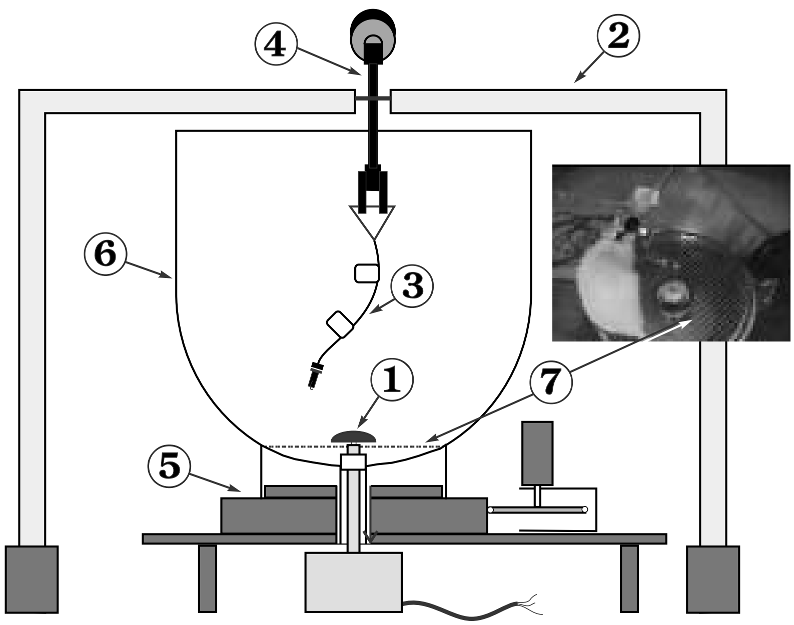
Animals

Male Sprague-Dawley rats (weighing 300 - 350 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and housed under controlled lighting temperature. Food and water were available ad libitum. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 86-23) and was approved and monitored by the Institutional Animal Care and Use Committee of Illinois State University.

Surgery

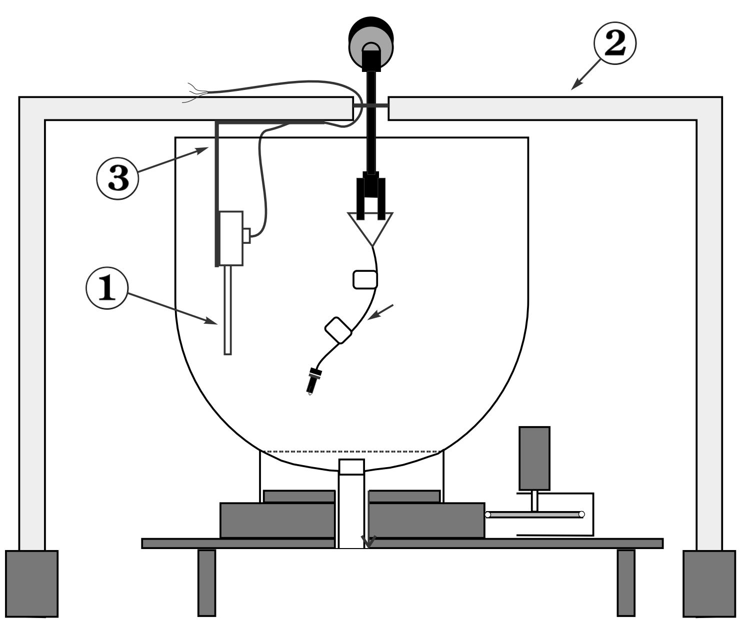
A bipolar stimulating electrode (MS 303/2, Plastics One, Roanoke, VA) was implanted in the brain reward system to activate ascending DA neurons according to Garris et al. (9) with some modification. Briefly, animals were anesthetized with Equithesin (3 ml/kg i.p.) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Body temperature was maintained at approximately 36°C by a Deltaphase Isothermal Pad (Braintree Scientific, Braintree, MA). Skin and muscle layers on the skull were retracted and holes were drilled for placement of reference, working, and stimulating electrodes. Two holes were also drilled into the skull to thread surgical screws for securing the dental cement. Stereotaxic coordinates were based on a flat skull between bregma and lambda using the atlas of Paxinos and Watson (14). Anteroposterior (AP) and mediolateral (ML) coordinates were referenced from bregma and dorsoventral (DV) coordinates referenced from dura.

The stimulating electrode was initially located just dorsal to the substantia nigra/ventral tegemetal region (-5.6 AP, +0.8 ML, -7.0 DV) and incrementally lowered until a signal, voltammetrically-identified as DA (15), was observed at a carbon-fiber microelectrode (16) implanted in the caudate-putamen (+1.2 AP, +2.0 ML, -4.5 DV). Extracellular DA was evoked by a 60 Hz, 0.4 s train of biphasic stimulus pulses (125 µA and 2 ms each phase) and measured by fast-scan cyclic voltammetry (17) using an EI 400 potentiostat (Cypress Systems, Inc., Lawrence, KS). A chloridized silver wire (18), situated in superficial cortex contralateral to stimulating and working electrodes, served as the reference and counter electrode for electrochemistry. After optimizing the location of the stimulating electrode to obtain a robust evoked signal, working and reference electrodes were removed and holes in the skull were filled with bone wax. The stimulating electrode was then cemented (Dentsply: Caulk, Milford, DE) in place. Animals were allowed at least two weeks for recovery before experimentation.



F1a

An experimental platform press was created for the Raturn by the manufacturer. It utilized the standard instrument table (2), tether line (3), counterbalanced arm (4), and turntable (5) on the Raturn system, and a modified bowl (6) with hole in the bottom for the press assembly. The animal walked on a wire-mesh floor (7) and could step on the platform press (1). The photograph shows an overhead view of a tethered rat circling the platform press at the center of the floor.



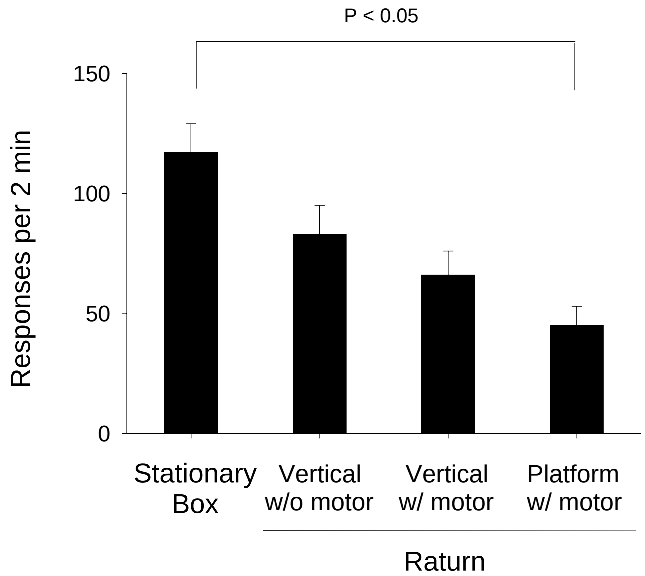
F1b

The same apparatus was then modified to remove the bottom press bar and replace it with an omnidirectional lever press assembly, mounted to the underside of the instrument table (2) with a special mounting bracket (3). The animal could reach up and press the lever (1) to activate the mechanism.

Contingent Intracranial Electrical Stimulation

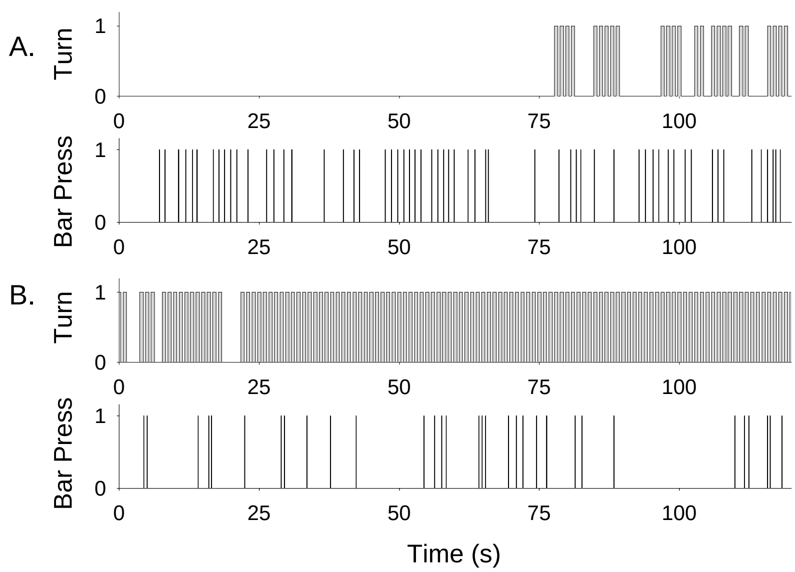
One lever press resulted in the application of a 60 Hz, 0.4 s train of biphasic stimulus pulses. Each phase was 1 ms in duration and either 50 or 100 µA in intensity, depending upon which current elicited maximum lever pressing. Pulses were generated by two stimulators (SD9 and S48, Grass Instruments, Quincy, MA), set to opposite polarity and synched together, and passed through a constant current device

F2



Response rates for the four conditions used to study ICS. The Stationary Box condition was the rectangular Plexiglas chamber equipped with the horizontal lever placed near the floor of one corner. For the Vertical w/o and w/ motor conditions, the omnidirectional lever was lowered in the containment bowl with the motor controller engaged and disengaged, respectively. The Platform w/ motor condition described the specially constructed platform press built into the floor of the containment bowl. Data are the mean+SEM (n = 4). Statistical analysis demonstrated a significant effect of lever press design on bar pressing rate ((F(3,12) = 8.09, P < 0.01). However, the only significant difference observed between designs was between Stationary Box and Platform w/ motor (P < 0.05) as indicated in the figure.

F3



Comparison of ICS and turning behavior for the two types of lever presses used with the Raturn™. Two figures are shown in each panel. The top figure is a record of optical sensor activation monitored every sec. One bar represents a single activation of either left or right sensor by turning behavior of the rat. The bottom figure is a record of lever presses monitored every 100 ms. One bar represents a single lever press, which resulted in application of a reinforcing train of pulses. Data in each figure are from a representative rat and were collected simultaneously. Panel A. Omnidirectional lever press. Panel B. Platform press.

(NL 800, Neurolog, Medical Systems, Great Neck, NY). A commutator (SL12C, Plastics One, Roanoke, VA) connected the constant current device to the freely moving animal in the Plexiglas™ box. The lever press was also converted to a voltage pulse (Stimulator 6012, Harvard Apparatus, South Natick, MA) and recorded by VCR (JVC HR-VP638U) using a magnetic recorder (PMC Recorder Model 200, A.J. Vetter Co., Rebersburg, PA).

Non-Contingent Intracranial Electrical Stimulation

Recordings of ICS stored on VCR tape were played back through the PMC Recorder. The output was used to trigger the Grass Stimulators and trains of pulses were delivered to the animal as described above.

Behavior

Animals were trained for ICS using the method of successive approximation (10). Bar pressing rates were compared under four conditions (***F1***). The first condition was a locally constructed, rectangular Plexiglas box, approximately 17 x 22 x 32 cm with metal rods (3 mm diameter) spaced 12 mm apart as the floor. A lever connected to a microswitch was placed near one corner approximately 25 mm from the floor and side. The other three conditions utilized the Raturn (Bioanalytical Systems, West Lafayette, IN). In two of these conditions, an omnidirectional lever (Model 80111, Lafayette Instruments, Lafayette, IN) was lowered vertically into the animal bowl of the Raturn and ICS was monitored with the controller for the base motor turned on or off. For the fourth condition, a specially constructed platform press, which activated a microswitch when depressed vertically, was placed in the floor of a modified animal bowl. The platform was spherical in shape with a diameter of approximately 2.5 cm. The Raturn was also used to monitor behavior during non-contingent intracranial electrical stimulation.

Statistical Analysis

Where applicable, data are expressed as the mean+SEM and n is the number of animals. Significance was tested by one-way ANOVA and post-hoc comparisons were performed by the method of least squares with a Bonferroni correction (19). The significance level was set at $P < 0.05$.

Results and Discussion

Contingent Intracranial Electrical Stimulation

F2 compares response rates recorded during ICS for the four conditions used in the study. The Plexiglas box with the horizontal lever clearly supported the most robust response rates, which were on average about twice those recorded for the other conditions. This result indicated that activation of the omnidirectional lever or the platform press required more work than the horizontal lever in the Plexiglas box. In support of this notion, the latter lever appeared to be more easily triggered by hand compared to the former. Response rates were not significantly different when measured in the Raturn with the omnidirectional lever whether the motor controller was engaged or disengaged. This result suggested that the Raturn with the motor controller engaged did not substantially affect the behavior of bar pressing. Responses rates measured with the platform press in the Raturn were also similar to those measured with the omnidirectional lever.

Despite the similarity in response rates measured in the Raturn, there were marked differences in the behaviors associated with ICS. As shown in ***F3***, rats activating the omnidirectional lever to obtain reinforcing electrical stimulation appeared focused on the lever and locomoted very little during bar pressing (Panel A). Even during the times of locomotion measured toward the end of the recording, response rates were normal. The behavior documented by the record of optical sensor activation is qualitatively similar to that observed for animals in the Plexiglas box during ICS. During trials of robust bar pressing under this condition, an animal may transiently wander from the lever. However, animals return quickly and resume ICS without the requirement for priming pulses. In sharp contrast to the omnidirectional lever, rats were very active during ICS using the platform press (Panel B). It appeared that rats fortuitously activated the lever as they locomoted within the containment bowl. As a result, the rats may have associated reinforcing intracranial electrical stimulation with locomotion rather than activation of the platform press. Such intense locomotor activity was never observed in the Plexiglas box or in the Raturn using the omnidirectional lever during ICS.

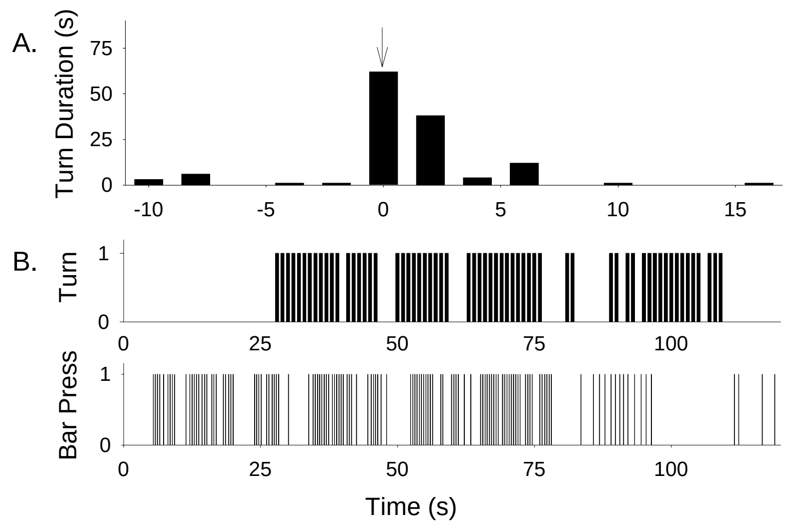
Non-Contingent Intracranial Electrical Stimulation

F4 describes locomotor activity during experimentally-applied stimulation. Similar to the yoked-control design, a recording of bar pressing collected during ICS was used as the pattern for intracranial electrical stimulation. As shown in Panel A, animals were largely inactive during the baseline measurement in the Raturn. However, play-back of a two-minute recording of ICS dramatically increased locomotion. Activity remained high for the two minutes following play-back but was quickly extinguished thereafter.

Panel B describes the individual activation of optical sensors along with the bar press record for experimentor-applied stimulation. Interestingly, sensors were not immediately activated despite robust electrical stimulation during play-back. After a lag period of about 25 seconds in the example shown, locomotor activity was dramatically increased and remained relatively constant at a high level during the duration of the stimulation. The lack of optical sensor activation early in the record belied no change in behavior. On the contrary, in excellent agreement with our previous work (10), non-contingent electrical stimulation elicited hyper-exploratory behavior characterized by excessive weaving, head-bobbing, rearing, and sniffing. Apparently, the Raturn was insensitive to these movements. The hyper-exploratory behavior was sustained until giving way to the rat running in place, activity readily documented by the sensor activation record.

F4

Behavior evoked by non-contingent intracranial electrical stimulation and measured with the Raturn™. All data were collected in a representative rat. Panel A. Turn duration. After a 10 minute baseline measurement, a 2 minute recording of bar pressing, previously recorded for one rat during ICS in the Plexiglas box, was played backed to another rat (see arrow at time 0 min). Prior to baseline measurement, the rat was equilibrated to the Raturn for approximately 30 minutes. Total (left and right) turn duration in seconds was calculated every two minutes. Panel B. Stimulus train record and turning behavior. Sensor activation is shown in the top figure and the record of bar presses is shown in the bottom figure. See Figure 3 for details.



Conclusion

The present results indicate that contingent and non-contingent intracranial electrical stimulation elicit distinct behaviors in the rat. In sharp contrast to experimental applied stimulation, which evokes a profound behavioral activation, rats actively bar pressing to obtain reinforcing electrical stimulation are primarily stationary. It is interesting to speculate that the observed behavioral activation is related to brain levels of dopamine, which are increased during non-contingent but not contingent electrical stimulation (10). One proposed function of dopamine is to modulate the behavior reactivity of an animal to ensure an appropriate response to external stimuli (11). The proper motivational state of an organism may be necessary for the response to novelty (12) or the prediction of reward (13), other functions recently associated with dopamine as well.

We also demonstrate that the Raturn is well suited for quantifying differences in behavior elicited by contingent and non-contingent intracranial electrical stimulation. As such, the Raturn modified with the omnidirectional lever is suitable for assessing behavioral during other operant paradigms. Indeed, the capability of the Raturn for combined neurochemical and behavioral monitoring provides a powerful tool for investigating the neurobiology of food reward (20-21) and drug self-administration (22-25), for example. Furthermore, we propose that the utility of the Raturn would be substantially improved by the addition of a sensor capable of monitoring vertical movement similar to that occurring with rearing and head-bobbing.

Acknowledgements

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