PROOF-OF-PRINCIPLE OF ED-DBS (EXPERIENCE-DRIVEN DEEP BRAIN STIMULATION) IN THE HEMIPARKINSON RAT MODEL

by

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ACADEMIC ETHICS AND INTEGRITY STATEMENT

I, Betül Tamer, hereby certify that I am aware of the Academic Ethics and Integrity Policy issued by the Council of Higher Education (YÖK) and I fully acknowledge all the consequences due to its violation by plagiarism or any other way.

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ABSTRACT

PROOF-OF-PRINCIPLE OF ED-DBS (EXPERIENCE-DRIVEN DEEP BRAIN STIMULATION) IN THE HEMIPARKINSON RAT MODEL

Parkinson's Disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopamine (DA) neurons in the substantia nigra (SN). The loss of DA leads to debilitating motor symptoms, such as tremors, rigidity, and bradykinesia. Deep brain stimulation (DBS) is considered state-of-the-art in the treatment of motor symptoms in advanced PD. The standard practice is to stimulate the subthalamic nucleus (STN) or internal globus pallidus (GPi) continuously via surgically implanted electrodes. However, continuous administration of DBS conflicts with the firing patterns of nigrostriatal DA neurons because these neurons generate rapid phasic DAergic signals in response to specific experiences, such as the presentation of a reward-predicting stimulus (S) or an unexpected reward (S*). This conflict may be one of the reasons for only moderate treatment efficacy of DBS. In our Boğazici University Scientific Research Projects (BAP) project, we hypothesize that treatment outcomes are enhanced if DBS is given acutely, in an experience dependent (ed) fashion that is tailored to specific experiences of the subject, involving S-S* contingencies *(Experience-driven (ed)-DBS to improve motor symptoms in the hemiparkinson rat model* under grant number 15981). Towards a test of this hypothesis, we will a) integrate DBS with operant conditioning in which experiences can be completely controlled, and b) determine the value of ed-DBS for the brain. In this thesis, we took initial steps towards achieving these aims. Specifically, we integrated the operant setup with DBS and tested the viability of our methods with two rats, one hemiparkinson rat and one control. In this thesis work, we report data which show support for the success of our behavioral training procedures, surgical approach, and integration of a conditioned inhibition procedure with ed-DBS.

Keywords: Variable Interval Schedule, Discrimination Learning, Hemiparkinson Rat, 6-Hydroxydopamine, Apomorphine-Induced Rotation.

ÖZET

HEMİPARKİNSON SIÇAN MODELİNDE ED-DBS (DENEYİME DAYALI DERİN BEYİN STİMÜLASYONU) METODUNUN KANITLANMASI

Parkinson hastalığı (PD), substantia nigrada bulunan dopaminerjik nöronların aşamalı ölümüne dayanan nörodejeneratif bir hastalıktır. Dopamin kaybı, titreme, rijidite gibi yıkıcı motor semptomlara yol açar. Derin beyin stimulasyonu (DBS), ileri derece Parkinson hastalığının motor semptomlarının tedavisi için bir teknoloji harikası kabul edilmektedir. Standart uygulama, subtalamik çekirdeği (STN) veya internal globus pallidus'u (GPi) cerrahi olarak implante edilmiş elektrotlar aracılığıyla sürekli olarak uyarmaktır. Fakat, sürekli DBS uygulaması, nigrostriatal dopaminerjik nöronlarının çalışma paternleri ile çelişir; çünkü bu nöronlar, ödülü öngören bir uyaranın (S) veya beklenmedik bir ödülün (S*) sunumu gibi belirli deneyimlere yanıt olarak hızlı fazik dopaminerjik sinyaller üretir. Bu çelişki, DBS'nin yalnızca orta düzeyde tedavi etkinliği sunmasının nedenlerinden biri olabilir. Boğaziçi Üniversitesi Bilimsel Araştırma Projeleri (BAP) destekli projemizde, DBS'nin, S-S* ilişkilerini kapsayan, öznenin spesifik deneyimlerine göre uyarlanmış, deneyime bağlı bir tarzda akut olarak verilirse tedavi sonuçlarını iyileştireceği hipotezini kurduk (Hibe no: 15981). Bu hipotezi test etmek için a) deneyimlerin tamamen kontrol edilebildiği edimsel koşullandırma ile DBS'yi entegre edeceğiz, ve b) beyin için ed-DBS'nin değerini belirleyeceğiz. Bu tezde, bu hedeflere ulaşmak için ilk adımları attık. Spesifik olarak, operant kurulumunu DBS ile entegre ettik ve yöntemlerimizin uygulanabilirliğini bir hemiparkinsonlu sıçan ve bir control ile test ettik. Bu tez çalışmasında, davranışsal eğitim protokollerimizin, cerrahi yaklaşımın ve koşullu bir inhibisyon prosedürünün ed-DBS ile entegrasyonunun başarısını destekleyen verileri rapor ediyoruz.

Anahtar Sözcükler: Değişken Aralıklı Pekiştirme Tarifesi, Kavrayış Yoluyla Öğrenme, Hemiparkinson Sıçan Modeli, 6-Hidroksidopamin, Apomorfinle İndüklenmiş Rotasyon.

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S*	Primary Reward (reinforcer)
S	Reward-Predicting Stimuli
R	Lever Press
S1	Tone
S2	Triple Cue Light
S3	Clicker
S1+S2	Tone+Triple Cue Light Compound Stimulus
S2+S3	Triple Cue Light+Clicker Compound Stimulus
S1+S3	Tone+Triple Cue Light Compound Stimulus

LIST OF ABBREVIATIONS

PD	Parkinson's disease
DA	Dopamine
SN	Substantia Nigra
DBS	Deep Brain Stimulation
STN	Subthalamic Nucleus
GPi	Internal Globus Pallidus
ed-DBS	Experience Driven-Deep Brain Stimulation
SNc	Substantia Nigra Pars Compacta
STR	Striatum
MSN	Medium Spiny Neurons
SNr	Substantia Nigra Pars Reticulata
GPe	Globus Pallidus Externus
hPD	Hemiparkinson
BÜHADYEK	Boğaziçi University Institutional Local Ethics Committee for the Use of Animals in Experiments
GS4	Graphic State 4
ECB	Environmental Control Board
RDY	Ready State
FIN	Finish State
GBL	Global State
FR	Fixed Ratio
VI	Variable Interval
ITI	Inter Trial Interval
MFB	Medial Forebrain Bundle
6-OHDA	6-Hydroxydopamine
APO	Apomorphine

1. INTRODUCTION

1.1 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disease characterized by dopaminergic neuronal death in the substantia nigra pars compacta (SNc) and striatum (STR), leading to the subsequent disruption of the basal ganglia as well as cortical and subcortical networks [1],[2], [3]. The loss of these neurons results in crippling motor and non-motor symptoms, including tremors, bradykinesia, dementia, and changes in olfaction, mood, and sleep [4].

James Parkinson first described PD more than 200 years ago in *An Essay on the Shaking Palsy*[4],[5]. Since then, it has become the second most prevalent neurodegenerative disorder after Alzheimer's disease, with an estimated 10 million people with PD worldwide [6]. Unfortunately, there is currently no cure for PD. Treatments are available, but have much room for improvement. Among them, pharmacologic treatment of PD is the first line of response and aims for dopamine replacement to alleviate motor symptoms [4]. However, it eventually requires more drugs, higher doses, and higher dosing frequencies to control the symptoms. Deep brain stimulation (DBS) is the most common surgical treatment for motor symptoms of PD [7],[8] in patients whose symptoms persist despite careful and appropriate medication management.

1.2 Deep Brain Stimulation

Deep brain stimulation is considered state-of-the-art for the treatment of motor symptoms in advanced PD. DBS involves electrical stimulation of the brain through surgically implanted uni- or bilateral electrodes connected to a neurostimulator (also called a pacemaker) [9]. The neurostimulator is implanted subcutaneously below the collarbone and sends electrical pulses continuously through the electrodes, the ampli-

tude, pulse width, and frequency of which are programmed via the neurostimulator. Stimulation parameters are individually adjusted based on patients' symptoms and needs [9].

The subthalamic nucleus (STN) and globus pallidus internus (GPi) are two targets that are approved to be used in the clinic and have been shown to help with the management of PD motor symptoms; the former reduces medication use more efficiently and the latter offers better dyskinesia suppression [10]. Another study showed that STN-DBS may help improve some cases in which patients respond well to levodopa treatment, but suffer from uncontrollable fluctuations [11].

1.3 DBS and Motor Movement

DBS aims to compensate for the loss of dopaminergic input in the basal ganglia circuit, which controls motor movement through direct and indirect pathways [12]. Direct and indirect pathways exert their effects on movement control via opposing effects. In the direct pathway, excitatory dopaminergic projections from the SNc are relayed via D1 receptors on the striatal medium spiny neurons (MSNs). These MSNs innervate the GPi and substantia nigra pars reticulata (SNr), whose activity in turn disinhibit thalamocortical neurons and excite the motor cortex, leading to motor movement [12],[13]. Activation of the indirect pathway, on the other hand, results in the inhibition of motor activity. Dopaminergic input from the SNc is relayed via D2 receptors on striatal MSNs to the globus pallidus externus (GPe) and STN. Consequently, DA input to indirect pathway via D2 receptors leads to inhibition of this inhibitory pathway and results in increase in movement [12],[13].

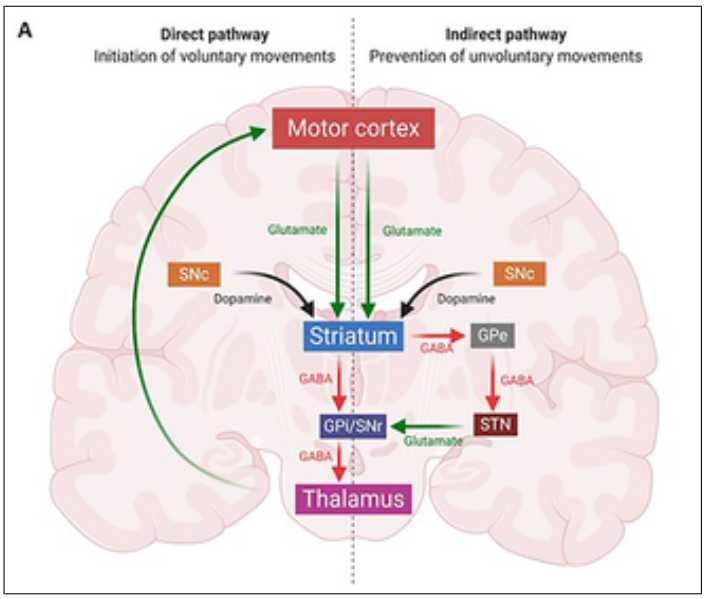

Figure 1.1: Direct and indirect pathways of basal ganglia motor circuit. The image was adapted from Ferrini et al., 2021 [12].

Degeneration of dopaminergic SNc neurons in PD leads to a lack of input into basal ganglia motor circuits. Lack of dopaminergic input leads to underexcitation of the direct pathway, which triggers movement. and increased inhibition of the indirect pathway, which leads to a reduction in movement [12],[13]. Overactivation of inhibitory indirect pathway in PD leads to symptoms such as difficulties in movement initiation, rigidity and slowed movements. STN-DBS is thought to exert its function by reducing this overactivation of the indirect pathway, thus increasing motor output. Studies have reported elevated activity of dopaminergic neurons in the SNc following STN-DBS treatment, in addition to reductions in levodopa medication dosage by approximately 50% [14],[15],[16].

1.4 Efficacy of DBS and the Need for Improvement

Randomized clinical trials have established the efficacy of STN-DBS [10], [11], [17], [18], [19]. Accordingly, DBS has been shown to improve PD motor symptoms by approximately 40% and help reduce levodopa intake by up to 50%. However, treatment outcomes may vary from patient to patient, and the reasons for these variations are yet to be elucidated.

One reason for unsuccessful DBS has been attributed to faulty electrode placement [20]. Literature [21] has shown that current implantation techniques utilizing stereotaxic frames or surgical robots yield an average precision in the range of 1-2 mm from the target area. However, the brain can also shift 2-4 mm during surgery. Such deviations may contribute to errors in precise targeting in up to 40% of DBS surgeries in general [21].

In addition to inaccurate targeting and electrode implantation, the continuous administration of DBS may also lead to a low efficacy in the treatment of symptoms. After surgery, stimulation parameters are fixed in a limited range (high/level/low) and stimulation involves no further adjustments based on neural activity in the target region. Such constant stimulation without feedback from the target region seems problematic with regards to the functioning and phasic firing patterns of dopaminergic neurons.

1.5 Phasic Firing of DA Neurons and S-S* Contingencies

DA neurons generate multiple responses based on the behavior they regulate, such as learning and goal-oriented behavior [22].These processes, in which DA neurons participate, involve an interaction between a person and the stimuli in the environment in a well-defined timely manner.Schultz [23],[24] showed that DA neurons of the SNc produce rapid, subsecond, phasic DA signals in response to novel stimuli and unexpected primary rewards (S*), as well as conditioned, reward-predicting stimuli (S), which are initially neutral but then become associated with S* through repeated pairing. Before learning occurs, DA signals respond to S*. Learning shifts this signal from S* to S (Figure 1.2). If S* is omitted, the signal is depressed.

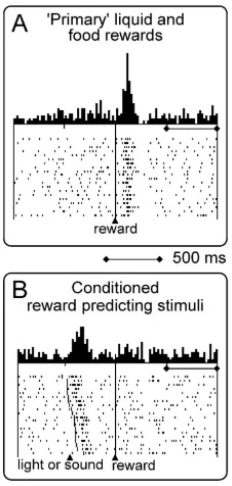

Figure 1.2: Time courses of DA signals in response to reward and (S*) and reward predicting stimulus (S). The images were adapted from Schultz, 2010 [24].

In response to a novel stimulus, an unexpected reward (S*), or reward-predicting stimulus (S), DA neurons in the SNc generate a single burst with an onset latency of $<$100 ms and a duration of $<$200 ms (Figure 1.2) [22],[24]. When S* is not received, the resulting depressions have onset latencies of 100-200 ms and durations of 200-300 ms. Reward-predicting stimuli (S) generate 10-100 impulses/s, which is slightly higher than background activity [22]. These fine adjustments in the generation of phasic DA signals precisely align the DA signals to incoming stimuli. As a result, learning of associations between S and S* occurs [23],[24].

Therefore, the continuous administration of DBS, which is intended to rescue a deficient DA system, does not comply with the impact of external stimuli that typically trigger DA release. The discordance between continuous DBS applications and normal functioning of dopaminergic SNc neurons might lead to disruptions in symptom control, resulting in only moderate treatment efficacy in DBS treatments.

1.6 Novelty

To improve DBS efficacy, we propose a novel method for DBS administration. We call this novel application experience-dependent (ed) DBS, which is based on acute stimulation of the STN in a way that is integrated with the specific experiences of the subject. To establish ed-DBS, this thesis completed the first objective of a BAP-funded larger project entitled Experience-driven (ed)-DBS to improve motor symptoms in the hemiparkinson rat model (grant number 15981). In the BAP project, we hypothesize that treatment outcomes are enhanced if DBS is given acutely, in an experience-dependent (ed) fashion that is tailored to specific experiences of the subject, involving S-S* contingencies. To the best of our knowledge, this has not been tested before. As the initial steps of testing this hypothesis, this thesis integrated the operant setup with DBS and tested the viability of the proposed methods with two rats, one hemiparkinson rat (hPD), and one control.

2. MATERIALS AND METHODS

2.1 Animals

6 naïve adult male Wistar albino rats (ages at the start of the study: 9 weeks) were used in this study. Animals were kept in transparent cages (two rats per cage) in a temperature (range: 21.1 - 29.9°C) and humidity-controlled animal room (range: 24% - 81%) with a controlled daily light cycle (12h dark/12h light; lights on at 8:00 a.m.). Upon their arrival to the animal room, all animals went through a 15-day adaptation period during which they had ad lib access to water and food (Optima Yem).

Following the adaptation period, 2 of the animals were spared as control animals for body development/weight monitoring and had ad lib access to water and food. 4 animals were provided with ad lib access to water except for during experiments and were fed with food according to individual food restriction diet schedules that are discussed in detail in the next section. They also received reinforcer (45 mg dustless precision pellets; Bio-Serv) during behavioral training sessions and the daily received amount was deducted from their diet every day. Among 4 experimental rats, 2 of them (Rat #2 and Rat #4) completed all experimental procedures. The other 4 rats (including 2 experimental rats and 2 weight control rats) did not recover from the surgery. All experiments and animal care conditions were approved by Boğaziçi University Institutional Local Ethics Committee for the Use of Animals in Experiments (BÜHADYEK). Timeline of the study is shown in Table 2.1 below.

Table 2.1: Timeline of the experiments.

Procedure	Food Restriction Schedule	Instrumental Training	Discrimination Training - S1	SURGERY	Recovery from Surgery	Apo-induced Rotation Test	Recovery Discrimination Training - S1	Threshold Test	Compound Training	Discrimination Training - S3	Summation Tests
Animals **Involved**	#1-#2-#3-#4	#1-#2-#3-#4	#1-#2-#3-#4		#2-#4		#2-#4	#4	#2-#4	#2-#4	#2-#4
Number of Days(d)/ Sessions(s)	15 d	16 d	30 s- 23 s 23s - 21 s		32 d - 25 d		22 s - 48 s	3 s	22 s - 59 s	7 s - 7 s	3 s - 3 s

2.2 Food Restriction Schedule and Pre-Feeding

Following the adaptation period, the experimental animals started following a food restriction schedule after a 24h of food deprivation. Accordingly, each experimental animal was fed 70% of their free feeding amount (5 g of rat food per 100 g of body weight) until they reached 90% of their free feeding body weight. Afterwards, individual food restriction schedules were continuously adjusted depending on each animal's changing body weight; the feeding amounts varied between 70% and 90% during the study. Once the operant training sessions started, daily reinforcer amount received by each rat during the training was deducted from their daily feeding amount.

To prevent neophobia during behavioral training sessions, rats were familiarized with the reinforcer beforehand. Preceding the operant training, the rats were fed with 5 g of precision pellets provided in a dish on the floor of their home cage for 3 consecutive days. This amount was deducted from their daily feeding amount.

2.3 Experimental Setup

Operant trainings took place in a Skinner Box (Habitest, Coulbourn Instruments; 36 cm height, 31 cm width, 26 cm depth) equipped with two retractable levers (H23-17RA, Coulbourn Instruments), a clicker (H12-05R, Coulbourn Instruments), two triple cue lamps (H11-02R, Coulbourn Instruments), a house light (H11-01R-LED, Coulbourn Instruments), a 4.5 kHz high-power tone module (H12-02R-4.5, Coulbourn

Instruments), a pellet feeder (H14-23R, Coulbourn Instruments), a magazine (H14-01R-SP04, Coulbourn Instruments), and a non-shock floor (H10-11R-TC-NSF, Coulbourn Instruments). As viewed on the left side of Figure 2.1, the ceiling, the left and the right walls of the chamber consisted of stainless steel while the rear wall and the front door consisted of clear plastic. The right wall of the chamber had two retractable levers and a triple cue light on top of each. All of these modules were separated by the magazine. The pellet feeder was placed outside of the same wall on top of the magazine. The tone module was on the upper left corner of the right wall. The clicker was located on the lower left corner of the left wall, which was diagonally across from the tone. Finally, the house light was placed on top of the left wall, at the center. Figure 2.2 is another representative image of the experimental setup with precise locations of each module, except for the house light. We custom-built a sound attenuating chamber for this study specifically. Hence, the Skinner Box was placed inside the sound attenuating chamber (60 cm height, 83 cm width, 50 cm depth) which was made of oriented strand board (10 mm OSB, Bauhaus), as seen in Figure 2.1. To minimize bacterial growth on the surface we coated the chamber with a water-repelling base, followed by black mat paint to prevent reflection of light. We covered the left and the right walls with sound attenuating foams and left an opening at the backwall small enough for air circulation during training sessions as well as for the cables to be able to exit the chamber.

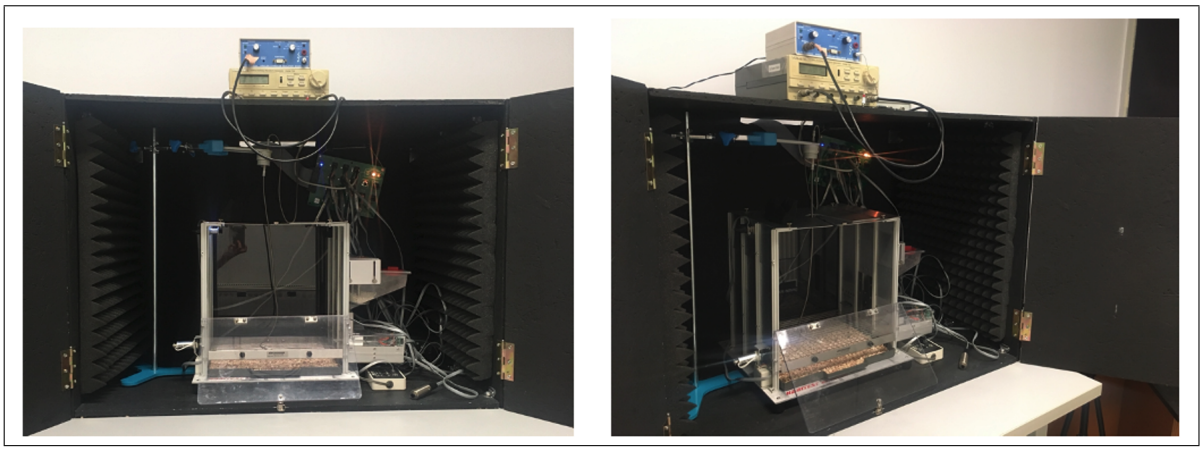

Figure 2.1: The Skinner Box used for operant trainings, placed in a sound-attenuating chamber.

Graphic State 4 RunTime Software (GS4.0, Coulbourn Instruments) recorded responses of the animals and operated the Skinner Box by sending commands via Habitest Linc (H02-01, Coulbourn Instruments) based on GS 4 protocols specifically created for distinct operant trainings. Habitest Linc was the crucial interface that enabled the communication between the Skinner Box and GS4 RunTime through the Environmental Control Board (ECB; H03-04, Coulbourn Instruments) by receiving and converting the responses of animals to GS4 language while simultaneously relaying the commands from GS4 RunTime to the Skinner Box (Figure 2.2). In other words, GS4 RunTime received "inputs" from the animal, which can be the presence or absence of a lever press, and sent related "outputs" in response to these inputs based on specific operant training protocols created on GS4.

Each experimental session started with a command from GS4 RunTime. This command signal traveled through Habitest Linc and reached the ECB. Depending on the command, ECB sent the signal to a specific Skinner Box module via connection cables between ECB and the Box. For instance, GS4 RunTime marked the start of each session by turning the house light on. In this case, GS4 RunTime sent a command signal to Habitest Linc, which was to be relayed to ECB. House light module in the Skinner Box received this signal from ECB via the connection cables between the module and ECB. As a result, the house light was on. When electrical stimulation was involved during an experimental session, the signal that triggered stimulation delivery was dependent on and produced by the animal's response. When the animal performed a lever press that was eligible for triggering stimulation delivery, this behavior was first carried from retractable lever module to ECB as an input signal. After that, the signal was first relayed to Habitest Linc and then to GS 4 RunTime, where its eligibility to trigger stimulation delivery was evaluated by the protocol. If the animal had pressed the lever on the "right time", the output signal for ed-DBS delivery was generated by GS4 RunTime and sent back to ECB via Habitest Linc. The signal was then relayed to TTL Trigger (custom made by Prof. Burak Güçlü). TTL Trigger initiated the process for ed-DBS delivery by activating 5 MHz Arbitrary Waveform Generator (Model 75A; Wavetek, San Diego, CA). The signals from the Arbitrary Waveform Generator were relayed to the isolated voltage-to-current converter (Model 2200; A-M Systems, Sequim,

WA, USA) in turn to generate biphasic pulses. Finally, the animal received ed-DBS via stimulation electrodes (Figure 2.2).

In response to commands coming from GS4 RunTime, the animal showed a behavior or not. In our experimental sessions, we trained the animals to show lever press as an input signal for GS4 RunTime. The signal that marked the presence of this behavior was received by retractable levers and relayed to ECB through connection cables. From there, the lever press input signal was transferred to Habitest Linc and then reached to GS4 RunTime. Depending on the presence or absence of a lever press (or input), GS4 RunTime sent the related command that was pre-determined in the protocol back to Skinner Box through Habitest Linc - ECB - connection cables - related module in the Box route (Figure 2.2). In addition to receiving inputs and sending specific outputs, in other words, operating the Box, GS4 RunTime recorded each input/output signals together with their exact timings.

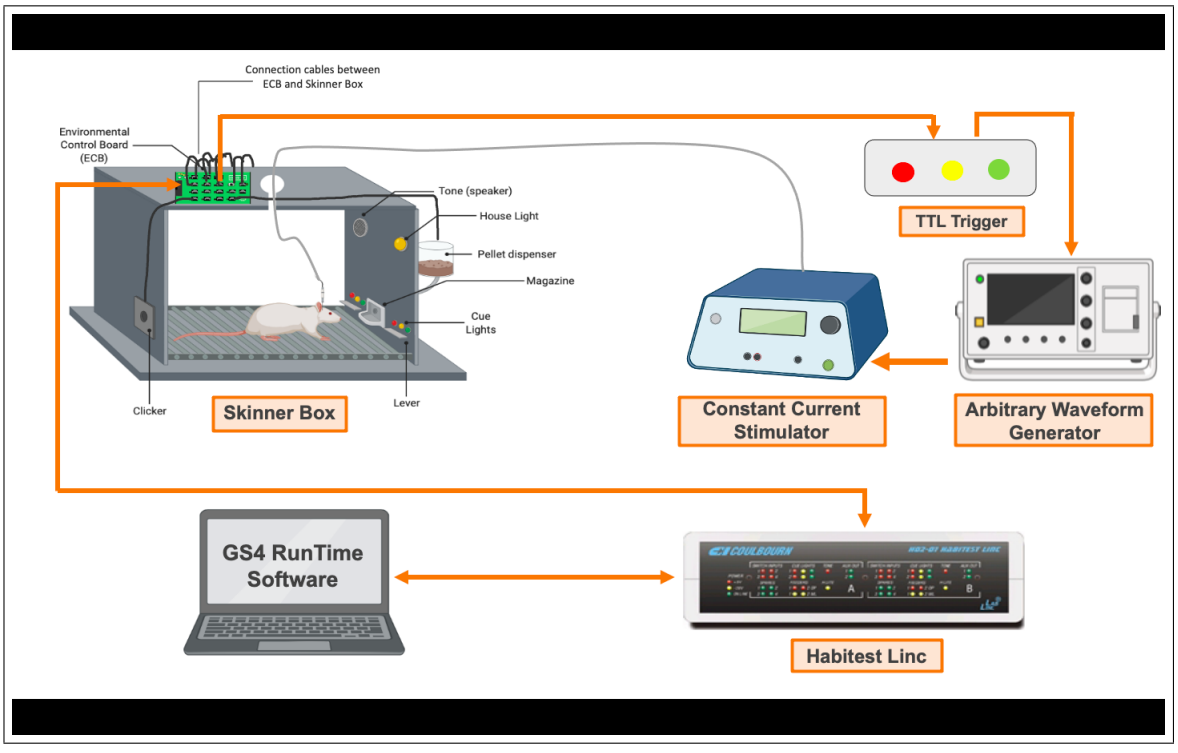

Figure 2.2: A conceptual presentation of the experimental setup used for operant trainings and summation tests.

2.4 Operant Trainings and GS4

Operant trainings involved four training steps, namely, instrumental training, discrimination training [S1: R →S1* vs EXT], compound training [S1+S2: R →ed-DBS vs no US], and discrimination training [S3: R →S1* vs EXT]. Our goal during operant trainings was to train the animals to press the lever (R) in order to receive S1* (reinforcer pellets) or ed-DBS when they were exposed to various stimuli, either S1 stimulus (the tone) and S3 (the clicker) or a S1+S2 (the tone and the triple cue light conjointly). In order to reach our goal, we created separate protocols on GS4 (Figure 2.3).

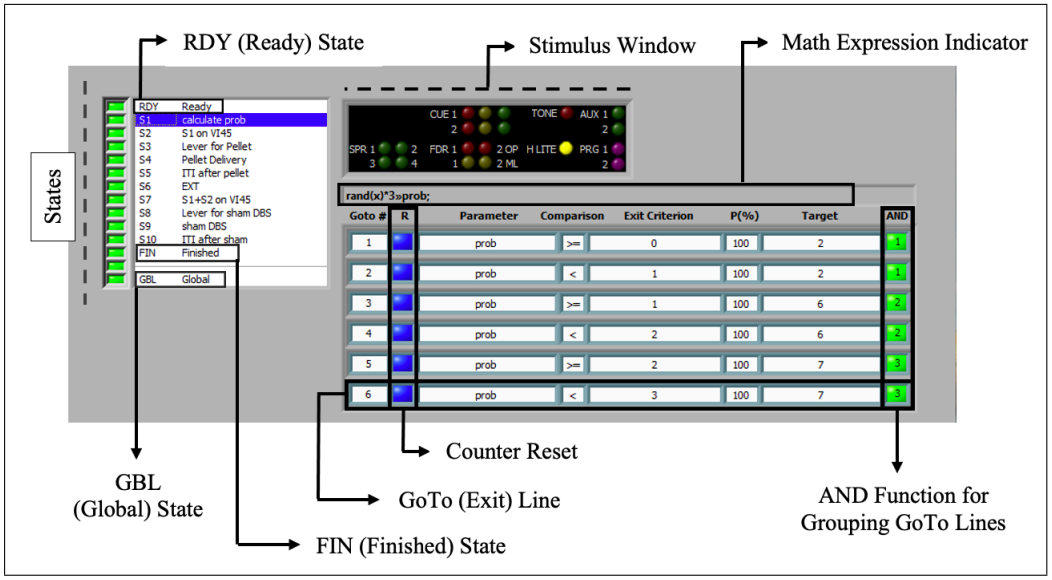

Figure 2.3: The Protocol Builder Screen in Graphic State 4.

States: Defines different conditions that involve the collection of controls (parameter, exit criterion, comparison, P, target). States operate according to defined functions in each Exit Line (or GoTo) or a subject's response.

RDY (Ready) State: This state is used to define the status of the environment before a session begins. In other words, RDY state is the first state the protocol enters once it is started and lasts until it reaches the defined exit criterion before moving to State 1 (S1).

FIN (Finished): Marks the end of an experimental session and defines the state of the stimuli when a session is completed. For example, retractable levers could be extended or retracted; house light could be turned off, etc.

GBL (Global): State exit parameters and stimulus conditions specified in the Global State will remain in effect for the duration of an experimental session.

Exit Line (GoTo): Each line in the states that involves the collection of controls (parameter, exit criterion, comparison, P, target) that determine when a state will exit.

Stimulus Window: Each module that is connected to the ECB is shown in the Stimulus Window and is activated/inactivated by clicking on the module.

Math Expression Indicator: Registers, in other words, math functions written by the user using Math Expression dialog, appear on Math Expression Indicator. These expressions are used to calculate the value of the target Register upon state entry.

R - Counter Reset: Parameters specified in Exit Lines are calculated by internal or external counters. If R - Counter Reset is activated, counters start calculation from 0 every time the protocol enters the state. If R - Counter Reset is inactive, counters record where they left off last time the protocol left from the state and keep counting from the last value, instead of 0.

AND: Exit Lines (GoTo) are by default OR'ed together. As a result, when the protocol enters a state, the first Exit Line to fulfill its exit criterion will switch the state to its target. When AND function is activated, selected Exit Lines are conjoined, and they can control the protocol towards the next state only when all of the grouped Exit Lines reach their exit criteria. When conjoined Exit Lines have the same target, the protocol moves to their common target when each of the Exit Lines reach to their exit criterion. When these lines have different targets, the protocol moves to the target of the last Exit Line that reaches to its target.

2.5 Instrumental Training Protocols and Their Transcriptions in GS4

Instrumental Training consisted of Magazine Training, Fixed Ratio (FR) 1, FR 2, FR 5, FR 10, Variable Interval 30 seconds (VI 30s) and VI 45s trainings. Our goal was to train the animals to lever press in such a way that they would sustain this behavior in time.

2.5.1 Magazine Training

In order to familiarize the rats with the magazine where S1* was delivered, we ran 15-min magazine training sessions for 2 days before starting FR 1 schedule. Magazine training involved S1* delivery according to a Variable Time 20 seconds (VT 20s) schedule, independent of lever press (R) and stimulus. VT 20s schedule was based on a list created in Microsoft Excel [25], consisting of numbers between 1 - 120 and with an average of 20. GS4 randomly picked numbers from this list and operated the timing of S1* delivery accordingly.

Magazine training started with a 30-seconds 'Ready' state where only the house light inside the Skinner Box was on. We started the protocol immediately after placing the animal inside the box. Thus, 'RDY: Ready' state of magazine training protocol marked the beginning of a training session with the house light turned on and functioned as a 30 second-habituation period to the chamber (Figure 2.4).

Since only the house light was on during this state, only H LITE module was active on Stimulus Window, while rest of the modules were inactive. There was only one GoTo Line whose *Parameter* was set to sec, *Comparison* was adjusted to >=, and *Exit Criterion* was set to 30. Once this condition was satisfied, in other words, the counter hit 30 seconds, GoTo line commanded the protocol to move to *Target 1*, which meant State 1 ('S1: VT 20s'), with a probability of *100 (P%)*. We activated *Reset (R)* function for this state so that the protocol would reset the counter and start counting from 0 until 30 every time the protocol enters this state. *AND* function was not needed, so, it was inactive.

Figure 2.4: 'RDY: Ready' state of Magazine Training protocol in Graphic State 4.

After 'RDY: Ready', the protocol moved on to 'S1: VT 20s' where the protocol delivered S1* noncontingent on R and contingent on VT 20s schedule (Figure 2.5). When the protocol entered this state, GoTo line randomly picked a number from the variable time interval list (named VT20s) that was registered as the exit criterion of this state. Accordingly, when the counter reached the randomly assigned number, the protocol moved to Target 2 ('S2: Reinforcer'). We created a Math Expression on the Math Configuration Window (Figure 2.6) to simultaneously calculate the number of entries to this state. The expression *e+1»e* is shown on Math Expression line.

We benefited from the number calculated by Math Expression in the next state to terminate the protocol.

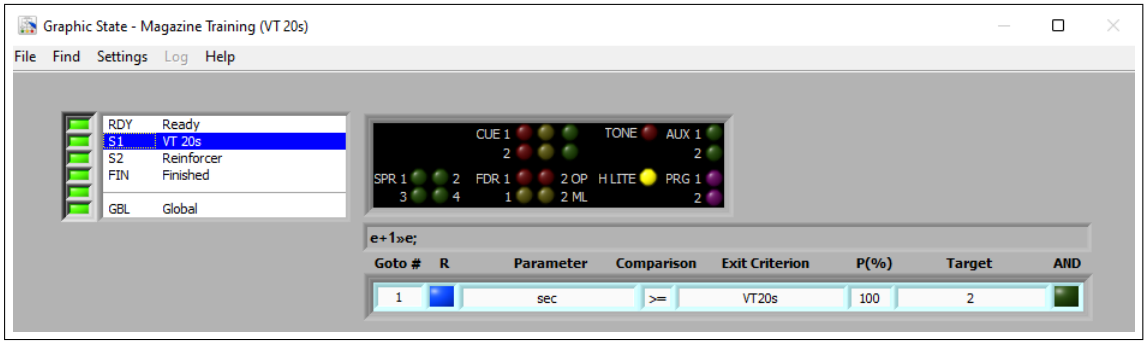

Figure 2.5: 'S1: VT 20s' state of Magazine Training protocol in Graphic State 4.

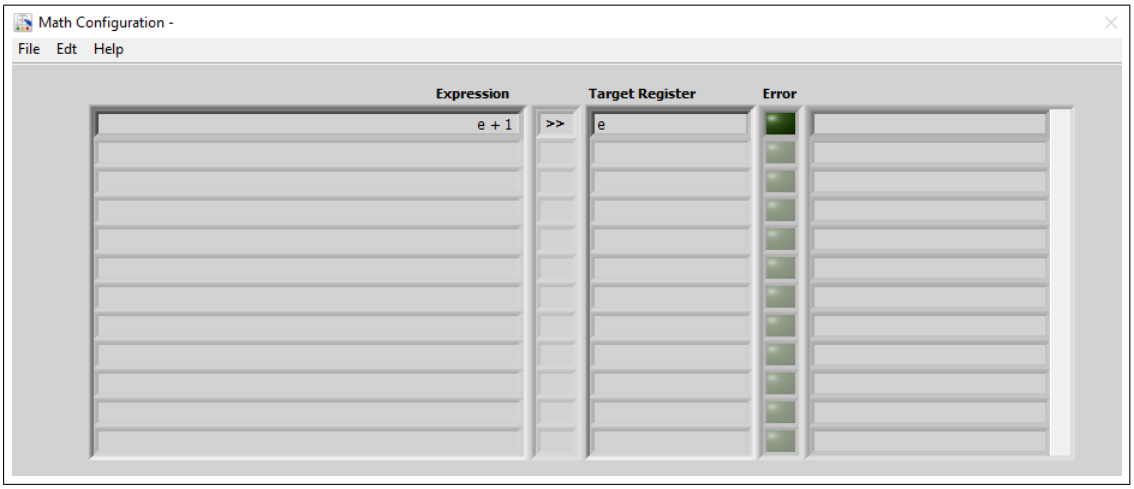

Figure 2.6: Math Configuration Window in Graphic State 4.

Following 'S1: VT 20s', the protocol moved to 'S2: Reinforcer', in which S1* was delivered (Figure 2.7). Since the pellet feeder and the magazine was active in this state, we activated FDR 1 and ML 1 modules on stimulus window for the feeder and the magazine light, respectively. GoTo #1 counted to 20 milliseconds, which was a sufficient time for the feeder to operate and drop one S1*. At the end of 20 ms, this line moved the protocol to next target state 'S1: VT 20s'. GoTo #2 monitored the number calculated by the Math Expression in 'S1: VT 20s to move the protocol to 'FIN: Finished' if the calculated number e reached to 150. The numerical equivalent of e in *Parameter* was updated in each entrance, in parallel with the numerical equivalent calculated in 'S1: VT 20s'. That is, when it was calculated as 150 in the previous

state, it would automatically be updated in 'S2: Reinforcer' as 150, thus, moving the protocol to 'FIN: Finished'.

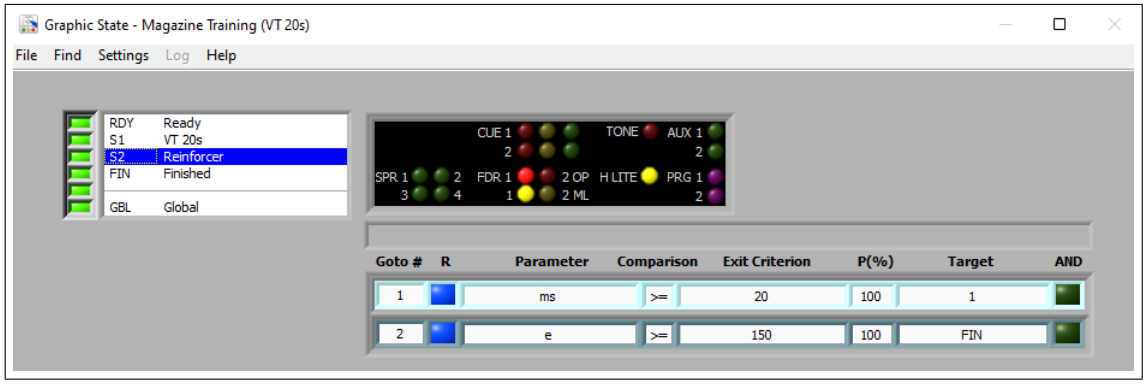

Figure 2.7: 'S2: Reinforcer' state of Magazine Training protocol in Graphic State 4.

We wanted this protocol to be terminated either after 15 mins or after 150 S1* delivery. When either one of these conditions were satisfied, the protocol jumped to 'FIN: Finished' (Figure 2.8). When the protocol entered this state, the house light turned off and marked the end of the training for the animal. Hence, there was no active module on the stimulus window. Similarly, there was no defined GoTo line since there was no condition to be fulfilled in this state. When the protocol entered this state, the training finished for the day and the animal was removed from the box. 'FIN: Finished' was the same in all other protocols.

Figure 2.8: 'FIN: Finished' state of Magazine Training protocol in Graphic State 4.

The last state we used in all protocols was 'GBL: Global' (Figure 2.9). 'GBL' is a state that always executes in the background, in line with the main sequence of other states. We used this state to keep track of the total time of the protocols. For instance, we wanted the magazine training protocol to run for 15 mins. Thus, this training protocol had a 'GBL: Global' that was set to move the protocol to 'FIN: Finished' when the counter reached to 15 mins.

Figure 2.9: 'GBL: Global' state of Magazine Training protocol in Graphic State 4.

2.5.2 Fixed Ratio (FR) Trainings

Following magazine training, we implemented FR trainings in four steps, namely, mult FR 1 - VT 20s, FR 2, FR 5, FR 10. We started a mult FR 1 - VT 20s schedule. The goal of this schedule was to train the rats for R while S1 was on to receive S1*. Thus, the rats received S1* immediately after 1 R, if R happened while S1 was on. Additionally, they received 10 S1* non-contingent on R under the VT 20s schedule. After 10 S1* delivery, the only way for the animals to receive S1* was to press the lever at least once when S1 was on. There was a 15 seconds Inter Trial Interval (ITI) following each S1* delivery in which there was no S1, and R did not result in S1*. To help the rats establish the association between the lever and S1* delivery, we also used an auto-shaper to deliver S1* when the rat was close to the lever or touched it. We stopped using the auto-shaper when the animal started lever pressing by itself. We ran mult FR 1 - VT 20s schedule for 3 days, and then FR 1 schedule without VT 20s for 2 days.

Once we observed that R was stabilized after 5 days, we moved on to FR 2 schedule where at least 2 R while S1 was on resulted in S1*. Depending on animals' performances, we then shifted to FR 5 and FR 10 schedules. That is, the animals were required to press the lever at least 5 and 10 times, respectively, to receive S1*. The number of sessions for each training protocol is shown in Table 2.2 below.

All operant training protocols, including FR trainings, started with a 1 min 'RDY: Ready'. In this state, only the house light was on, and the protocol moved to next target state at the end of 1 min (Figure 2.10).

Figure 2.10: 'RDY: Ready' state of mult - VT 20s - FR 1 protocol in Graphic State 4.

Following 'RDY: Ready', we expected the animal to respond to stimulus S1 (tone) in 'S1: Response' (Figure 2.11). In this state, the tone module was active on the Stimulus Window. We created three conditions, one of which was grouped inside by using *AND* function. Additionally, we used the same function we benefited in 'S1: VT 20s' of the magazine training protocol to calculate the number of entries to this state. Upon entry to this state, the protocol moved to *Target* 2 'S2: Reinforcer' if the animal pressed the lever at least once. At the same time, the number of entries to the state (e) was calculated. During the first 10 entries to the state, the protocol picked a random number from the variable time interval list (VT20slist) and delivered S1* at the end of the interval, independent of R. If the animal pressed the lever in the meantime, the protocol delivered S1* since in that case, one of the first two conditions had also been satisfied (S1* delivery as a result of at least 1 R). We inactivated *R Counter Reset* option for Exit Line 4 so that the protocol can continue tracking the number of entries to the state from where it left off. Starting from the 11th entry to

this state, the protocol did not deliver any more S1* independent of R and delivered S1* only when at least 1 R condition was satisfied. Since all of the grouped Exit Lines are required to fulfill their exit criterion in order to move to assigned target state, Group 1 lost its function after the first 10 entries to the state as Exit Line 4 failed to reach its exit criterion after 10 entries. GoTo #1 stood for a response on the left lever while GoTo #2 stood for a response on the right lever. Regardless of the Exit Line that moved the protocol to the next state, the protocol switched to 'S2: Reinforcer' following this state.

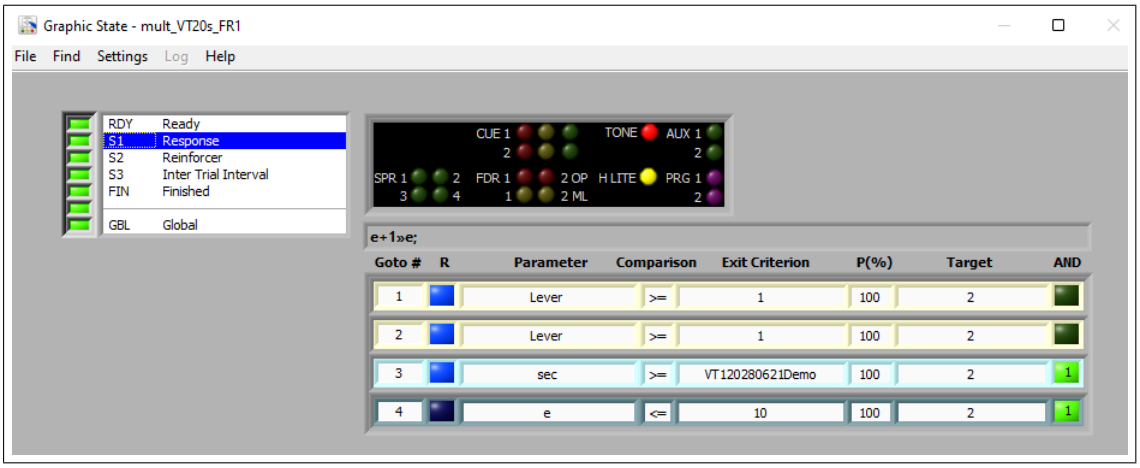

Figure 2.11: 'S1: Response' state of mult - VT 20s - FR 1 protocol in Graphic State 4.

'S2: Reinforcer' was the same as the one in the magazine training protocol. Except that mult - VT 20s - FR 1 protocol involved stimulus exposure. Hence, we activated TONE module in Stimulus Window so that the tone would be on during S1* delivery (Figure 2.12).

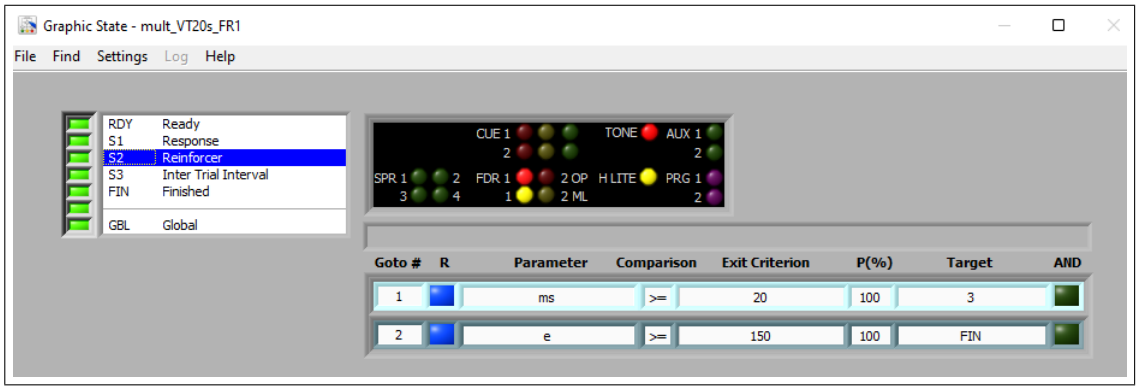

Figure 2.12: 'S2: Reinforcer' state of mult - VT 20s - FR 1 protocol in Graphic State 4.

Following S1* delivery, the protocol moved on to 'S3: Inter Trial Interval' in which the protocol waited for 15 seconds with only the house light on. Then, moved on to Target 1 'S1: Reinforcer' (Figure 2.13).

Figure 2.13: 'S3: Inter Trial Interval' state of mult - VT 20s - FR 1 protocol in Graphic State 4.

We wanted this protocol to run either for 2 hours or until 150 S1* was delivered. Thus, 'GBL: Global' of this protocol, just like the rest of all protocols, functioned as a counter in the background to terminate the training at the end of 2 hours (Figure 2.14).

Figure 2.14: 'GBL: Global' state of mult - VT 20s - FR 1 protocol in Graphic State 4.

After mult - VT 20s - FR1 training, we ran FR 2, FR 5, FR 10 training protocols. All other FR trainings followed the same GS 4 protocol as mult - VT 20s - FR1, except that there was no S1* delivery dependent on a VT 20s schedule. Instead, the animal was required to press the lever twice to receive S1* in FR 2 protocol (Figure 2.15). For the other protocols, the only difference was the number of required lever presses for S1* delivery. Thus, for FR 5 protocol, *Exit Criterion* was 5 and for FR 10 protocol, it was *10*.

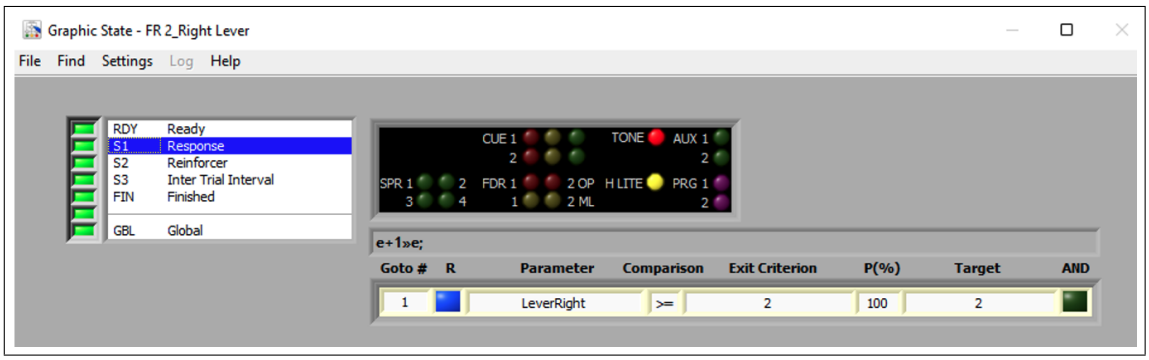

Figure 2.15: 'S1: Response' state of FR 2 protocol in Graphic State 4.

2.5.3 Variable Interval (VI) Trainings

Following FR schedules, we switched to VI schedules. We ran VI 30s schedule for 1 day, which involved a list of variable time intervals between 1 - 120 seconds and with an average of 30 seconds. In this schedule, the animals were required to press the lever at the end of a variable time interval which had started at the same time

as stimulus S1 (tone). In other words, S1 was continuously on during variable time intervals. These intervals were randomly assigned from the list created in Microsoft Excel [25] and determined the duration of the state they were embedded in. Each S1* delivery was followed by a 15-sec ITI where S1 was off, and R did not result in S1* delivery. Daily training sessions lasted either until the animals received 150 S1* or 2 hours.

After VI 30s schedule, we ran VI 45s schedule for 4 days. VI 45s schedule was based on the same principle as VI 30s schedule, except that the intervals varied between 30 - 120 seconds and had an average of 45 seconds. Daily training sessions lasted either until the animals received 150 S1* per session or for 2 hours. In total, instrumental training lasted for 16 sessions (Table 2.2).

Table 2.2: Timeline for instrumental training schedules for all animals.

	Magazine Training	Mult FR 1-VT 20s	FR 1	FR 2	FR 5	FR 10	VI 30s	VI 45s
Number of Sessions	2	3	2	2	1	1	1	4

Like all other protocols except magazine training, VI 30s and VI 45s protocols also started with a 1-min 'RDY: Ready'. Then, the protocol moved on to 'S1: VI 30s' (Figure 2.16) in which the protocol picked a random number from VI30s list and assigned this number as the duration of the state in each entry. During this state, stimulus S1 (tone) and the house light on the Stimulus Window were on. The protocol calculated the number of total entries into the state with *e+1»e* Math Expression seen on Math Expression Indicator and moved the protocol to 'FIN: Finished' when the number e hit 150. When the duration reached its exit criterion, the protocol moved on to 'S2: Response'.

Figure 2.16: 'S1: VI 30s' state of VI 30s protocol in Graphic State 4.

In 'S2: Response' (Figure 2.17), the protocol waited for a response from the animal, that is, lever press. At the end of a variable time interval in 'S1: VI 30s', the tone continued for an additional 15 seconds in 'S2: Response', and the animal was expected to press the lever once before 15 seconds period was over. If the animal had pressed the lever before the time was over, the protocol moved on to 'S3: Reinforcer' for S1* delivery. When there was no response from the animal (in other words, no R) at the end of 15 seconds, the protocol switched back to 'S1: VI 30s'.

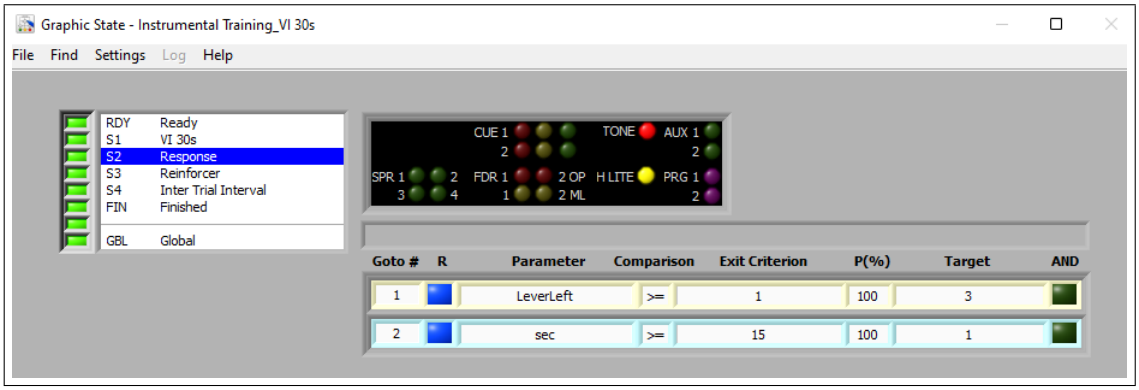

Figure 2.17: 'S2: Response' state of VI 30s protocol in Graphic State 4.

'S3: Reinforcer' (Figure 2.18) employed the same Exit Lines as FR protocols. The tone, the house light, and the feeder modules were on. *e+1»e* Math Expression calculated the number of entries to 'S3: Reinforcer', in other words, the number of S1* deliveries. When *e* reached to 150, the protocol jumped to 'FIN: Finished'.

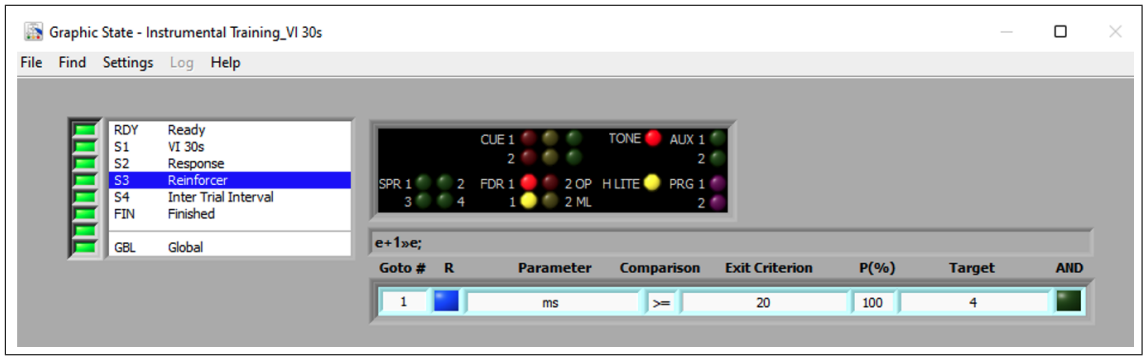

Figure 2.18: 'S3: Reinforcer' state of VI 30s protocol in Graphic State 4.

The animal entered 'S4: Inter Trial Interval' (Figure 2.19) in two conditions. When there was no R during 'S2: Response' and following S1* delivery, the protocol moved on to 'S4: Inter Trial Interval'. In this state, the tone was off, and the protocol went back to 'S1: VI 30s' until 150 S1* was delivered.

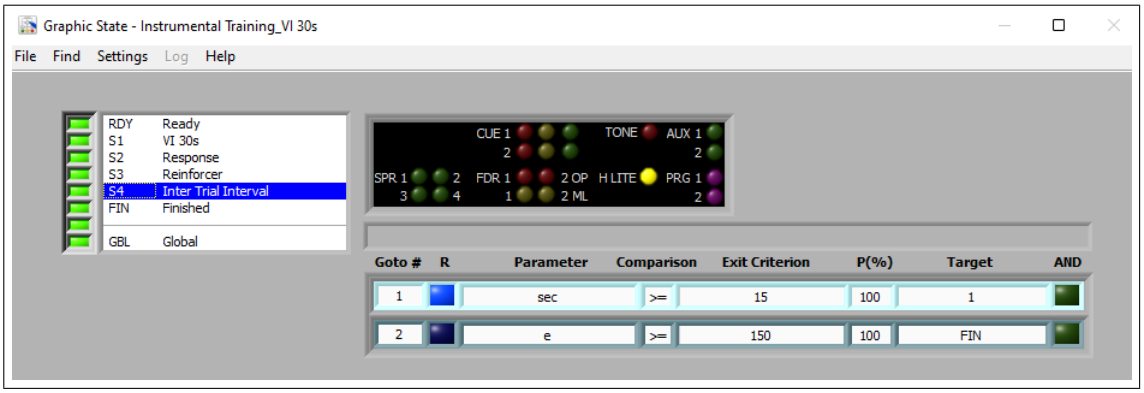

Figure 2.19: 'S4: Inter Trial Interval' state of VI 30s protocol in Graphic State 4.

VI 45s training differed from VI 30s training in that the former followed a different variable time interval list which consisted of numbers between 1-120 and with an average of 45. In other words, the variable time intervals in VI 45s training were longer than that were used in VI 30s training. As a result, VI 45s training protocol employed a different list (named VI45s) (Figure 2.20) but followed the same states as the VI 30s protocol during the rest of the training.

Figure 2.20: 'S1: VI 45s' state of VI 45s protocol in Graphic State 4.

2.6 Discrimination Training for S1: R →S1* vs EXT and Its Transcription in GS4

Instrumental training was followed by discrimination training [S1: R →S1* vs EXT]. In this training phase, we implemented a mult schedule which consisted of VI 45s and Extinction (EXT) schedules conjointly. The occurrence of each schedule was randomly picked by GS4, thus, both schedules had an equally likely chance to occur. VI 45s schedule consisted of randomly selected variable time intervals that ranged between 30 - 120 seconds and had an average of 45 seconds. During these intervals, S1 was on and R at the end of the interval resulted in S1* delivery. 15-sec ITI followed each S1* delivery, but R during ITI did not yield S1* although S1 was on. EXT schedule also consisted of randomly selected variable time intervals that ranged between 30 - 120 seconds but had an average of 60 seconds. During an EXT schedule, there was no S1, and R did not result in S1* delivery. Variable time interval lists for both schedules were generated in Microsoft Excel [25]. Daily training sessions lasted for 2 hours. Our success criterion for each animal to move onto the next training schedule was a Response Rate (RR; R/min) value. An RR value that was at least 5 times greater during VI 45s schedule compared to EXT schedule for 3 consecutive sessions was considered as successful [26]. Rats that reached a 5:1 VI 45s RR compared to EXT RR success criterion underwent stereotaxic surgery for the establishment of Hemiparkinson rat model or control model. Stimulation electrodes were also implanted in these surgeries. Since the timing of surgeries depended on each animal's individual performance, the

animals received discrimination training [S1: R →S1* vs EXT] for varying number of days (Table 2.3).

Table 2.3: Timeline for discrimination training [S1: R →S1* vs EXT] for each animal.

	Animal #1	**Animal #2**	**Animal #3**	**Animal #4**
Number of Sessions	30	23	23	21

Following 1-min 'RDY: Ready' with the house light on, the protocol always continued with 'S1: VI45'. In this state, a variable time interval was randomly chosen from the list (named VI45). The tone was on during this interval. At the end of the interval, the protocol switched to 'S3: Lever Press' (Figure 2.21).

Figure 2.21: 'S1: VI45' state of Discrimination training for S1 protocol in Graphic State 4.

The 'S3: Lever Press' was 15 seconds long. If the animals pressed the lever during this state, the protocol moved on to 'S4: Pellet Delivery' and a pellet was delivered. Starting with this protocol, we changed this state's name from "Response" to "Lever Press" to be able to interpret data more easily. At least 1 R during the state resulted in S1* delivery, while no R moved the protocol to 'S5: Probability' (Figure 2.22).

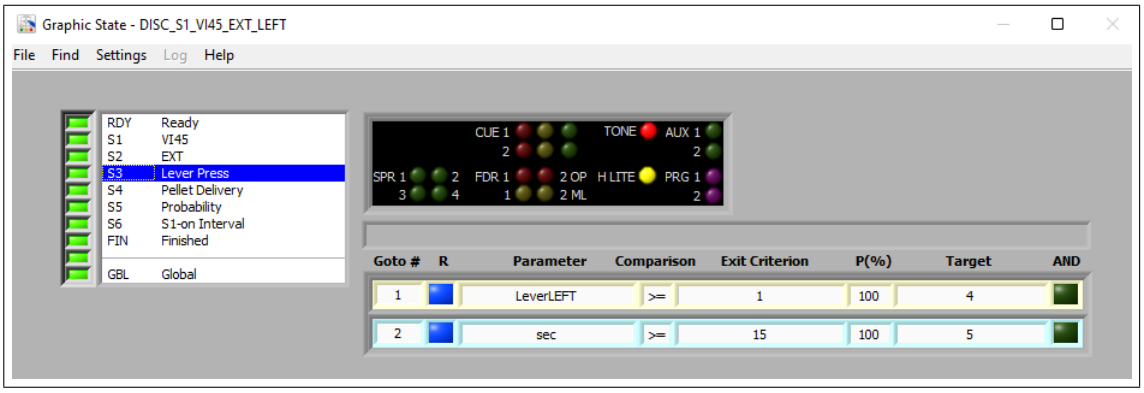

Figure 2.22: 'S3: Lever Press' state of Discrimination training for S1 protocol in Graphic State 4.

In the 'S4: Pellet Delivery' (Figure 2.23), we wanted to utilize a different feature of GS 4 and calculated the number of S1* deliveries by counting the number of entries to S1* delivery state. We replaced *e+1»e* Math Expression with an additional Exit Line that moved the protocol to 'FIN: Finished' when Entries was equal to or greater than 150. Until Exit Line #2 fulfilled its exit criterion, the protocol jumped to 'S6: S1-on Interval' following each S1* delivery.

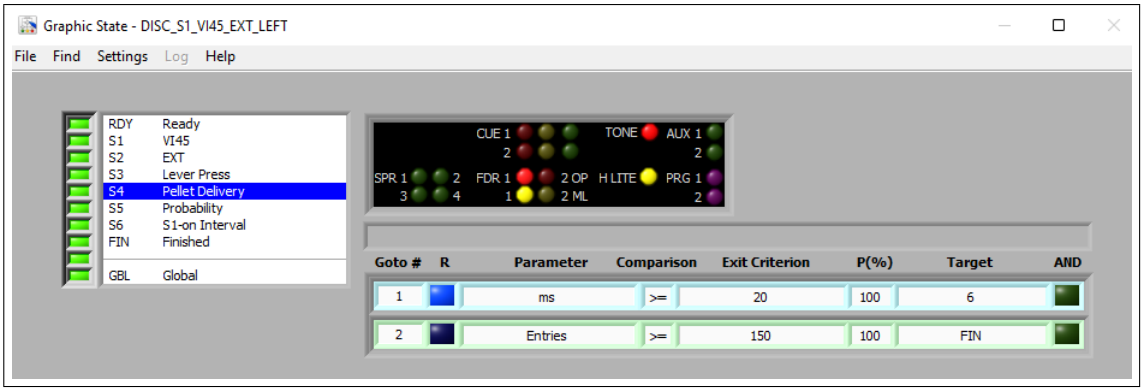

Figure 2.23: 'S4: Pellet Delivery' state of Discrimination training for S1 protocol in Graphic State 4.

In 'S6: S1-on Interval', the tone and the house light were on for 15 seconds. R did not result in S1* delivery. Later, the protocol switched to 'S5: Probability' (Figure 2.24).

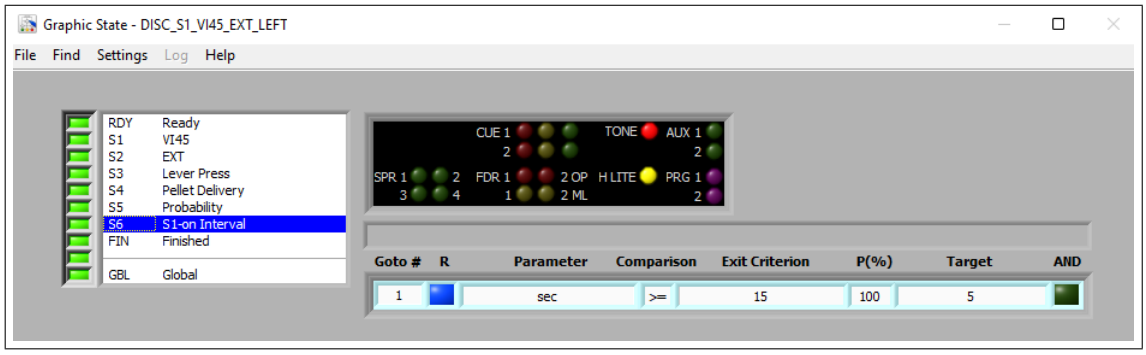

Figure 2.24: 'S6: S1-on Interval' state of Discrimination training for S1 protocol in Graphic State 4.

In discrimination training [S1: R →S1* vs EXT] training protocol, VI 45s and EXT schedules were equally likely to occur. By using Math Expression rand(x)»TRC shown in Figure 2.25, the protocol randomly decided which schedule to follow, and thus, which state (either VI45 or EXT) to enter at the end of each loop.

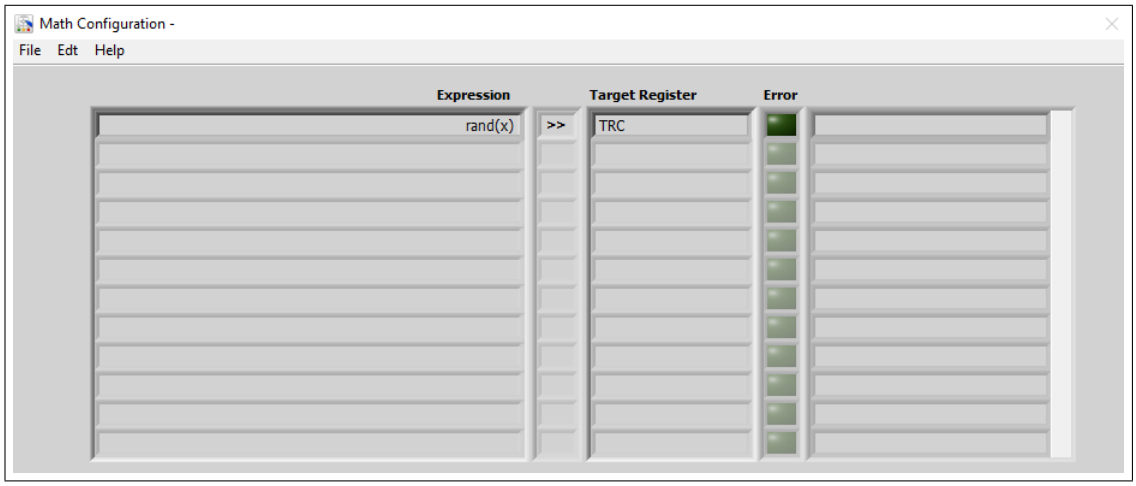

Figure 2.25: Math Configuration Window of Math Expression rand(x)»TRC used in Discrimination training [S1: R →S1* vs EXT].

When the protocol entered 'S5: Probability' (Figure 2.26), GS 4 employed rand(x)»TRC Math Expression and picked a random number. If the random number was greater than 0.5, the protocol jumped to 'S1: VI45'. If the random number was equal to or smaller than 0.5, the protocol followed 'S2: EXT'.

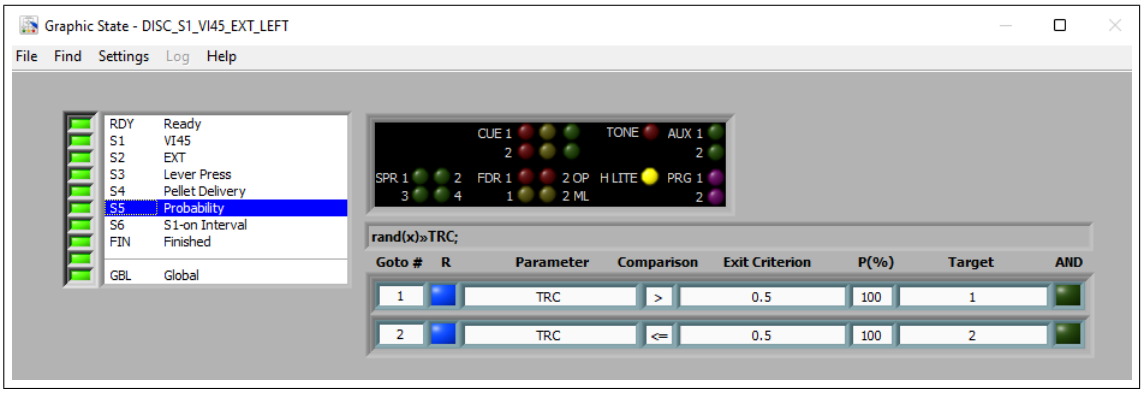

Figure 2.26: 'S5: Probability' state of Discrimination training for S1 protocol in Graphic State 4.

'S2: EXT' (Figure 2.27) lasted for variable time durations which were randomly assigned from a list named EXT60. This list was generated in Microsoft Excel [25] and consisted of numbers between 30-120 and with an average of 60. Just like 'S1: VI45', the protocol picked a random number from the assigned list and moved to the target state at the end of a variable time interval. However, there was no stimulus exposure, namely, no tone in this state and R did not result in S1* delivery whatsoever. Once satisfied, the Exit Line moved the protocol to 'S5: Probability'.

Figure 2.27: 'S2: EXT' state of Discrimination training for S1 protocol in Graphic State 4.

'FIN: Finished' and 'GBL: Global' in this protocol were the same as in our VI protocols.

2.7 Hemiparkinson Rat Model

We lesioned the medial forebrain bundle (MFB) with the neurotoxin 6-Hydroxydopamine (6-OHDA) to establish the hemiparkinson rat model. 6 animals underwent stereotaxic surgery in the order of their performance level. Table 2.6 presents the records of each animal regarding the surgery date, injection site, number of training sessions, and their status following the surgery.

Table 2.4: Surgery records (surgery date and the injection site), number of discrimination training [S1: R →S1* vs EXT] sessions, and status records of animals following surgeries. Since #5 and #6 were weight control animals, they were not trained.

Animal ID	**Surgery Date**	**Injection Site**	**Number of Training Sessions**	**Status**
#6	August 5, 2021	Right MFB	NA: weight control animal	Deceased
#5	August 9, 2021	Left MFB	NA: weight control animal	Deceased
#4	August 14, 2021	Left MFB	21 days	Alive
#2	August 18, 2021	Right MFB	23 days	Alive
#3	August 19, 2021	Left MFB	23 days	Deceased
#1	September 3,2021	Right MFB	30 days	Deceased

The animals were anesthetized for surgery with intraperitoneal (IP) injection of a ketamine/xylazine mixture (100 mg/kg/ml in ratio of 10:1). During the surgery, individual supplementary doses were applied as needed, based on each animal's pedal reflex and breathing pattern (injected between 0.05 - 0.1 ml supplementary dose for maintenance when number of breaths/min was more than 60-66). Some animals needed supplementary doses more frequently than others. #6 (311 gr) required nine supplementary doses whereas #5 (290 gr) and #3 (246 gr) required six supplementary doses. #1 (246 gr) was injected 4 supplementary doses. #2 and #4 stayed anesthetized during surgery with only two additional injections.

Body temperature was monitored, and the animals were covered with a blanket pad to keep their body temperature at 37+1°C. Their eyes were moistened with saline as needed. To prevent dehydration, we injected 1 ml saline intraperitoneally to each animal during surgery.

Following anesthetic induction, the animals were shaved on the head and placed in the stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The scalp was cleaned with 70% isopropyl alcohol prep pads (Kendall Webcol, USA), wiped with antiseptic solution (Batticon; Adeka, Turkey), and cleaned with 70% ethanol one last time. Right before and immediately after removing the scalp, we applied 1% Lidocaine HCI (Jetmonal; Adeka, Turkey) for topical analgesia. The skull was cleaned with 30% hydrogen peroxide (Merck, Germany) to precisely spot bregma and lambda. Injection and electrode implantation sites were marked according to following coordinates from Paxinos and Watson's rat atlas [27]:

6-OHDA injection coordinates for the left MFB: AP: -4.0 mm from Bregma, ML: +1.5 mm, DV: -8.5 mm from the skull

6-OHDA injection coordinates for the right MFB: AP: -4.0 mm from Bregma, ML: -1.5 mm, DV: -8.5 mm from the skull

Since the injection and the implantation sites were considerably close to each other, we opened a ▫ 2x3 mm2 craniotomy window for neurotoxin injection and electrode implantation. During the surgery, exposed brain was moistened as needed with saline. 30 minutes before 6-OHDA injection, the animals were intraperitoneally injected with 25 mg/kg desipramine HCI (Sigma-Aldrich, Germany) in order to prevent noradrenergic neuron death [28]. Following the removal of dura mater with a hooked needle, 12.5 µg of 6-OHDA (Sigma-Aldrich, Germany) dissolved in 3 µl of 0.9% saline with 0.1% ascorbic acid (Sigma-Aldrich, Germany) was injected into the left or right MFB counterbalanced across groups. Control animals received vehicle instead of 6-OHDA (Table 2.4).

For 6-OHDA and vehicle injections, we used a 33-gauge infusion cannula attached via tubing to a 2.5 ml glass syringe. We used an infusion pump (CMA 400 Syringe Pump; CMA Microdialysis, Kista, Sweden) to operate the glass syringe. The infusion rate was 0.5 µl/min. We left the cannula in the injection site for another 2 min following the injection to allow for the diffusion of the toxin or vehicle. Upon removal of the cannula, we proceeded with the next procedures to implant the stimulation electrode.

2.8 Implantation of Stimulation Electrode

For later applications of ed-DBS, we implanted a 26 gauge, 2-channel, bipolar, stimulation electrode made of stainless steel with a 0.5 mm-long bare tip and 1 mm gap between the two channels (model MS 303/3-A/Spc; PlasticsOne, Virginia, USA) in the left or right subthalamic nucleus (STN) of animals depending on their lesion hemisphere. Animals with the left MFB lesion had the electrode implanted into the left hemisphere while animals with the right MFB lesion had the electrode implanted into the right hemisphere (Figure 2.42). The electrode was placed into the STN according to coordinates from Paxinos and Watson's rat atlas [27]:

Electrode implantation coordinates for the left STN: AP: -3.8 mm from Bregma, ML: +2.4 mm, DV: -8.0 mm from the skull

Electrode implantation coordinates for the right STN:AP: -3.8 mm from Bregma, ML: -2.4 mm, DV: -8.0 mm from the skull

After electrode implantation, we covered the craniotomy window with bone wax during the first surgery (#6). Then, we switched to hemostatic sponge (Gelita-Spoon; Gelita Medical, The Netherlands) soaked in saline to provide a barrier between the cap and the tissue, for the remaining five surgeries. We covered the skull with a cap made of dental acrylic (Lang Dental, Illinois, USA) to hold the electrode in place and drilled 3 to 4 additional holes on the skull for anchor screws for rats (MF-5182; BASi, USA)

that hooked the cap onto the skull surface. The electrode was embedded in dental acrylic as well except for the plug on top of the socket. We used a dust cap to protect the plug.

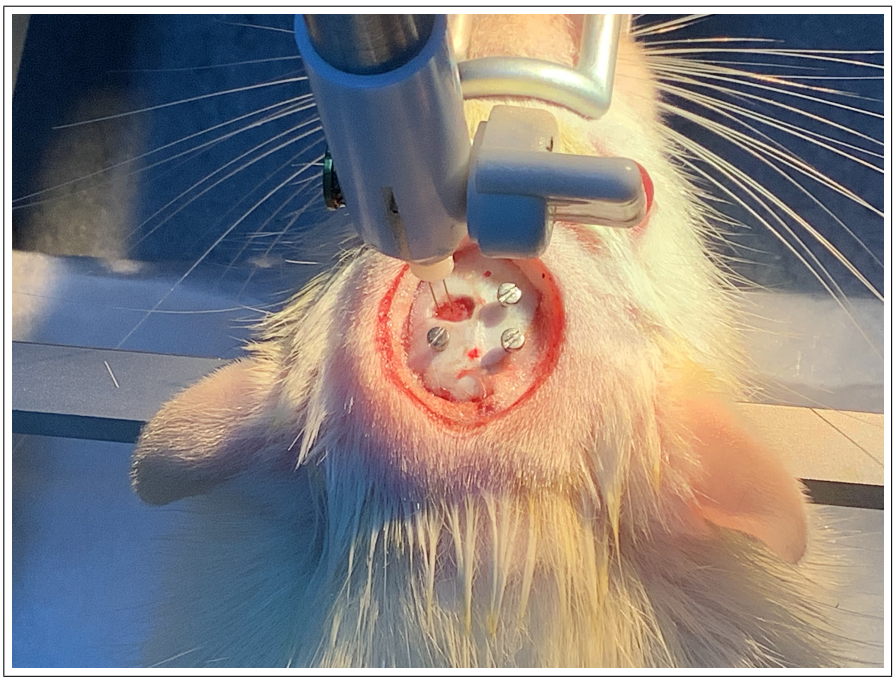

Figure 2.28: Implantation of stimulation electrode into the left STN of the animal.

2.9 Post-Operative Care

We treated all animals on the puncture site with Neosporin (Johnson & Johnson, New Jersey, USA), starting immediately after the surgery and continued as needed during the recovery period. Animals were placed in a clean cage with bedding and warming pads, and kept under a lamp following the surgery. They were kept in the lab and monitored until vigorous movements were observed, then transferred to the animal holding room. On the following days, wounds and behavior were observed and treated as needed. 2 of the animals (#2 and #4) recovered from the surgery and proceeded with the study until the end. The remaining 4 were found deceased the next day.

All animals, except #4, were treated with subcutaneous Meloxicam sodium hydrate (M3935; Sigma-Aldrich, Germany) injection ranging between 0.04 ml - 0.06 ml (5 mg/kg/ml dissolved in saline). 2 days after the surgery, #4 developed severe pain in the animal room and was treated with 0.05 ml of ketamine/xylazine mixture (10:1) in 0.5 ml saline followed by 0.05 ml Meloxicam in 0.2 ml saline, subcutaneously. He was ready to continue the trainings after a 36-days recovery period. #2 fully recovered and continued the trainings after 32 days. All animals were fed ad lib during the recovery period.

2.10 Apomorphine-Induced Rotation Test

Following a recovery period (#2: 32 days; #4: 36 days), #2 and #4 underwent apomorphine-induced rotation tests to verify the lesions in vivo. The test took place in a square open field arena (60 x 60 x 40 cm) and was recorded using a camera (acA1300-60gc; Basler, Germany). Recordings were manually scored afterwards. The tests consisted of three open field exposure that took place every other day. Each test lasted for 1 hour. The animals were transferred to lab in their home cages and waited there for at least 30 minutes for habituation to the environment prior to testing. The lights in the room were kept constant (upper right corner ▫ 15.07 lux; upper left corner ▫ 16.14 lux; lower right corner ▫ 29.06 lux; center ▫ 32.29 lux; lower left corner ▫ 33.37 lux). The animals continued ad lib feeding during Apomorphine-induced Rotation Test sessions.

On the first day, the animals received an intraperitoneal injection of 0.2% ascorbic acid (99%; A92902; Sigma-Aldrich, Germany) solution as vehicle. On the second test day, they received a low-dose of 0.05 mg/kg/ml apomorphine (>98.5%; A4393; Sigma-Aldrich, Germany) dissolved in 0.2% ascorbic acid solution. On the final day, they received a high-dose of 0.5 mg/kg/ml apomorphine solution. Only #2 received an additional 1 mg/kg/ml APO solution following the high dose test. The procedure and the justification for the additional dose is explained in the next paragraphs. We waited for 1 minute after each injection and then placed the animal into the center of

open field to start the test.

Intake of drugs that stimulate DA receptors, such as apomorphine, results in the induction of rotational behavior by acting on supersensitive DA receptors in the damaged neostriatum [29]. Accordingly, we manually counted the number of tight rotations in the open field arena for 1 hour. To verify the lesion in vivo, we expected to see tight contraversive turns (contralateral to the lesion) which constitute >80% of the tight rotations [29],[30].

Before surgery, #2 had been planned to be an experimental animal and received 6-OHDA injection into the right MFB. However, this animal did not display any tight turns following the high dose of APO. To examine whether #2 had a lesion too small to display turning behavior following 0.5 mg/kg, we also tested an additional dose (1 mg/kg), 3.5 hours after the high dose testing in the same day.

At the end of the study, we repeated APO-induced rotation test with the same doses. (Test 1: spontaneous turn with vehicle; Test 2: low dose injection with 0.05 mg/kg APO; Test 3: high dose injection with 0.5 mg/kg). Test scores of each animal are reported in the Results section.

2.11 Recovery Discrimination Training for S1: R →S1* vs EXT

Following stereotaxic surgeries, recovery period, and Apomorphine-induced rotation tests, the animals underwent mult VI 45s - EXT training as before the surgery. This was the same training schedule as the one that all animals had gone through preceding the surgery. The goal of this training was to bring the animals back to previous performance levels. Daily training sessions lasted for 2 hours. Our success criterion for this training was a baseline 5:1 performance for three consecutive days for VI 45s RR compared to EXT RR. Thus, it took a different number of sessions for each animal to reach their baseline performance before moving to the next procedure (Table 2.5).

After the first 4 sessions, we observed that the animals pressed the lever during VI 45s component approximately as many times as during EXT component. To increase the distinction between VI 45s and EXT, we provided the animals with a longer pause time in which there was no stimulus and no S1* delivery. Accordingly, we added 15 seconds to each variable time interval durations in EXT list. As a result, starting in the fifth session of recovery discrimination training [S1: R →S1* vs EXT], the animals waited for an additional 15 seconds every time they entered EXT component.

Table 2.5: Timeline for Recovery Discrimination Training [S1: R →S1* vs EXT] for #2 and #4.

	Animal #2	**Animal #4**
Number of Sessions	22	48

2.12 Habituation to Wire and Adjustment of Current Amplitude

The animal was placed in a transparent cylinder tube (30 cm diameter, 45 cm height) 20 mins before the adjustment of the current amplitude for habituation. The animal stayed in this cylinder tube during the adjustment of current amplitude (Figure 2.29). The animal was stimulated with an initial stimulation intensity of 0.5 V [31],[32] and the intensity was increased in 0.25 V steps [33]. Each step lasted approximately 10 seconds. During each step, the animal was monitored for dyskinesia assessment, which involved orofacial, axial, front limb, and locomotive dyskinesia [34]. The stimulation intensity that induced dyskinetic behavior was taken as the threshold and 90% of which was administered to the animal during compound training [S1+S2: R →ed-DBS] sessions.

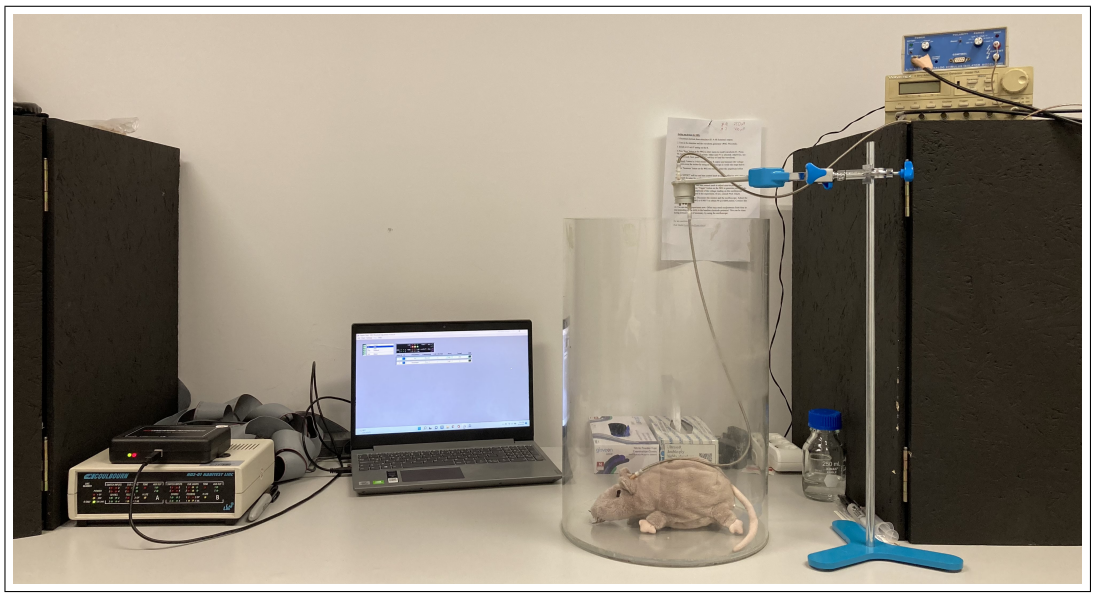

Figure 2.29: The setup used in current amplitude adjustment sessions.

During the stimulation assessment, we used an auto shaper-controlled protocol to stop and start current administration in between stimulation steps. We managed the protocol manually via auto shaper. Accordingly, 1 auto shaper input during 'S1: DBS' moved the protocol to 'S2: Stop'. Since the stimulator was connected to the ECB through Cue 2, we activated Cue 2 on Stimulus Window in 'S1: DBS'. 'S2: Stop' prevented DBS administration until another auto shaper input that moved the protocol back to 'S1: DBS' (Figure 2.30).

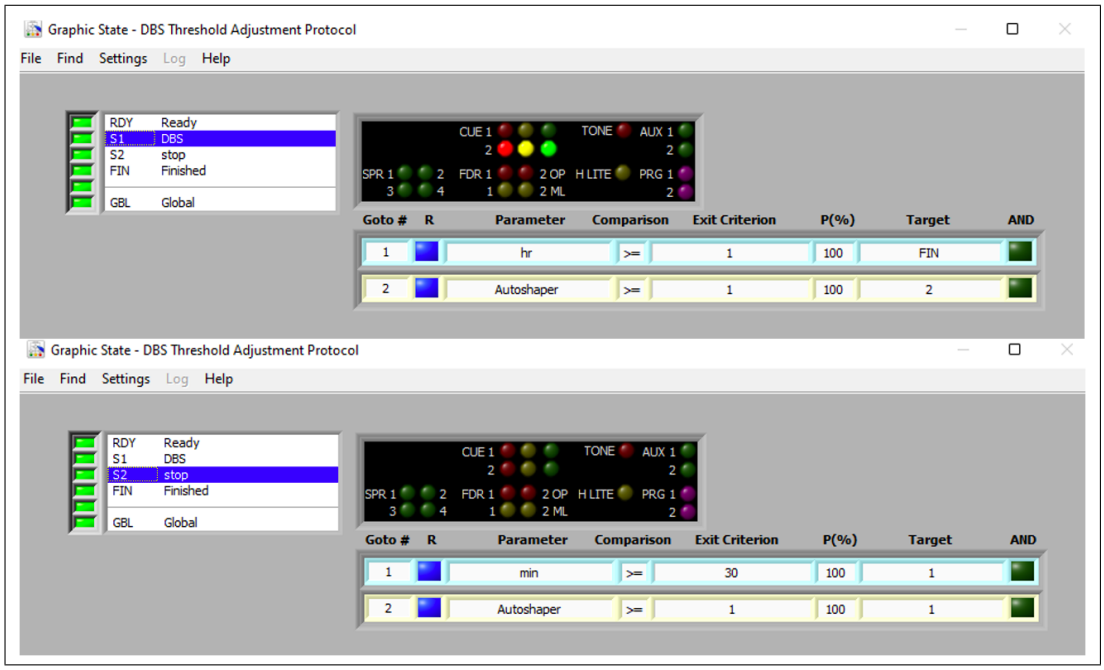

Figure 2.30: Protocols used in current amplitude adjustment sessions.

2.13 Compound Training for S1+S2: R →ed-DBS and [S1+S2: R →no S1*] and Their Transcriptions in GS4

Following recovery discrimination training [S1: R →S1* vs EXT] and stimulation threshold test, the animals started compound training [S1+S2: R →ed-DBS] or [S1+S2: R →no S1*] sessions. This training phase consisted of three components and the use of a novel stimulus S2 (triple cue light) together with stimulus S1 (tone), in addition to stimulus S1 alone and EXT. Component 1, named S1-on VI 45s, followed a VI 45s schedule as explained before. Component 2, named EXT, was the EXT schedule we also ran in discrimination training [S1: R →S1* vs EXT]. In component 3, named S1+S2-on VI 45s, the animal was presented with two stimuli conjointly as a compound stimulus. Accordingly, both S1 tone and S2 triple cue light was on in this component under a VI 45s schedule, which used the same variable time interval list as the one in component 1. In this condition, when the animal was exposed to a compound stimulus, R at the end of a variable time interval produced S2* delivery, namely, ed-DBS, or no US. Ed-DBS parameters were established based on the behavioral response of #4 to stimulation as discussed in the previous section and were in line with the literature

[31]. Ed-DBS that was administered to #4 consisted of 500 ms trains of symmetric 60 µs biphasic current pulses at 130 Hz. The current intensity was 90 µA (0.900 V) and was administered for 500 ms following R. The baseline amplitude on the stimulator was 0.1 mA/V. The offset on the stimulator was adjusted to 0.18 V.

Control animal #2 received no S1* instead of ed-DBS. Component training [S1+S2: R →no S1*] protocol involved the same procedures as [S1+S2: R →ed-DBS] protocol, except that there was no stimulation (no S2* delivery). The electrodes of #2 got broken during the first session of his compound training [S1+S2: R →no S1*]. As a result, he completed rest of the procedures without the stimulation electrodes and an acrylic cap. For all animals, the occurrence of the three conditions were equally likely and randomly selected by the protocol. Daily training sessions lasted 2 hours. To move onto the next procedure, we expected the Response Rate during S1-on VI 45s to strike 5:1 rate compared to EXT for at least three consecutive sessions. Each animal had their own timeline depending on their performances to move onto the next procedure, which was discrimination training [S3: R →S1* vs EXT] (Table 2.6).

Table 2.6: Timeline for Compound Training [S1+S2: R →no S1*] for #2 and [S1+S2: R →ed-DBS] for #4.

	Animal #2	**Animal #4**
Number of Sessions	22	55

Compound training [S1+S2: R →ed-DBS] consisted of three components that resulted in different outcomes depending on the animal's response following the tone stimulus (S1) alone, the tone and the triple cue light stimuli (S1+S2) conjointly, and no stimulus (EXT). Immediately after the 1-min 'RDY: Ready' with only the house light on, the protocol entered 'S1: calculate prob' in order to randomly select and employ one of the three conditions (Figure 2.31). All states in both [S1+S2: R →ed-DBS] and [S1+S2: R →no S1*] protocols followed the same rules in GS4, except for the one that delivered ed-DBS in [S1+S2: R →ed-DBS] protocol.

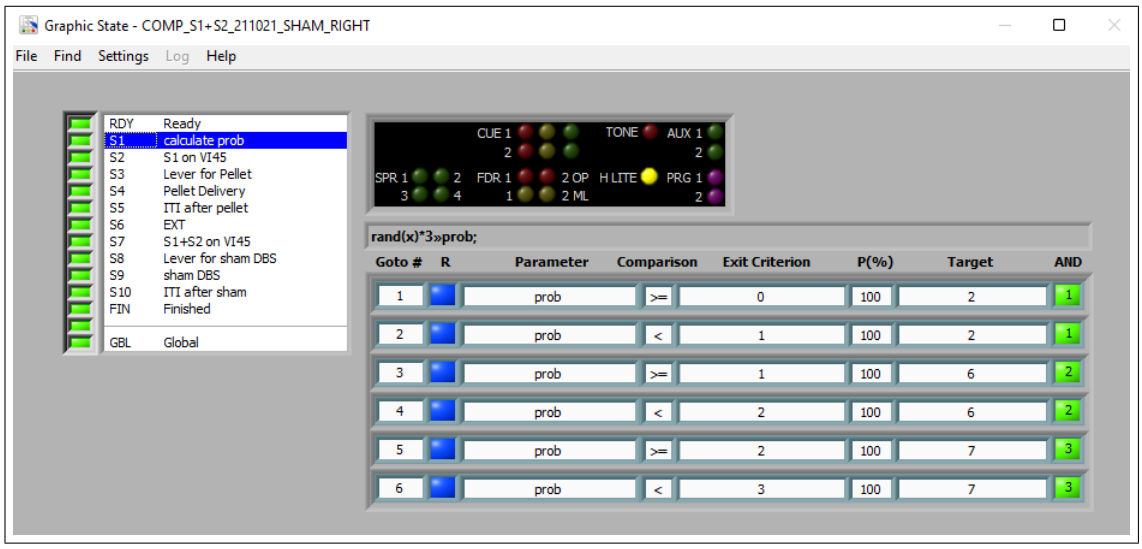

Figure 2.31: 'S1: calculate prob' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

In this state, we benefited from the Math Expression *rand(x)*3»prob* to generate a random number between 0 - 3 (Figure 2.32).

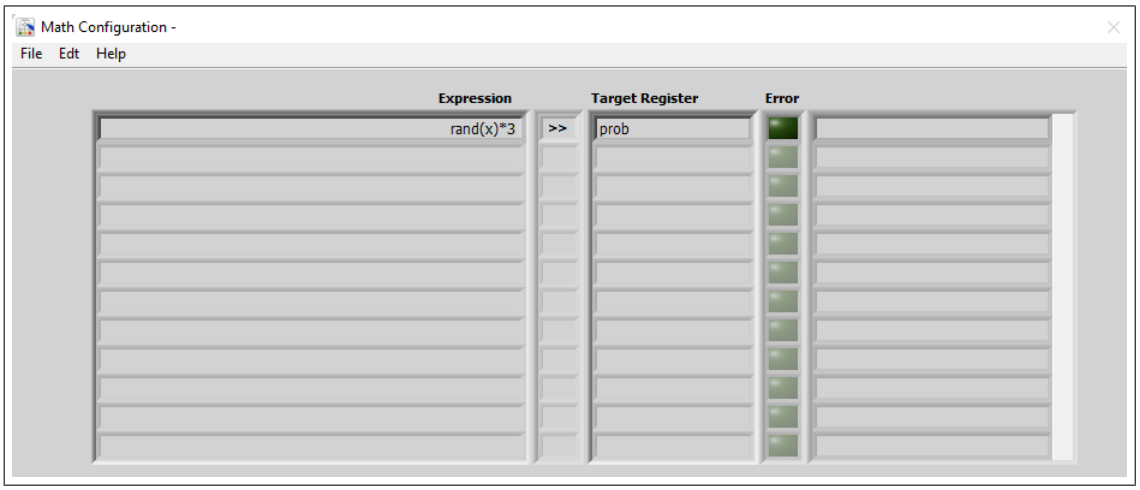

Figure 2.32: Math Configuration Window of Math Expression *rand(x)*3»prob* used in Compound Training [S1+S2: R →ed-DBS] and [S1+S2: R →no S1*].

Next, we assigned ranges to each condition between 0 and 3. Depending on the random number's range, the protocol jumped to a specific condition's state. The three conditions and their ranges are illustrated in Figure 2.30. If *rand(x)*3»prob* Math Expression generated a number which was less than 1 and equal to or greater than 0,

the protocol moved on to 'S2: S1 on VI45'. If the generated number was less than 2 and equal to or greater than 1, the protocol continued with 'S6: EXT'. If the number was less than 3 and equal to or greater than 2, the protocol switched to 'S7: S1+S2 on VI 45' (Figure 2.33).

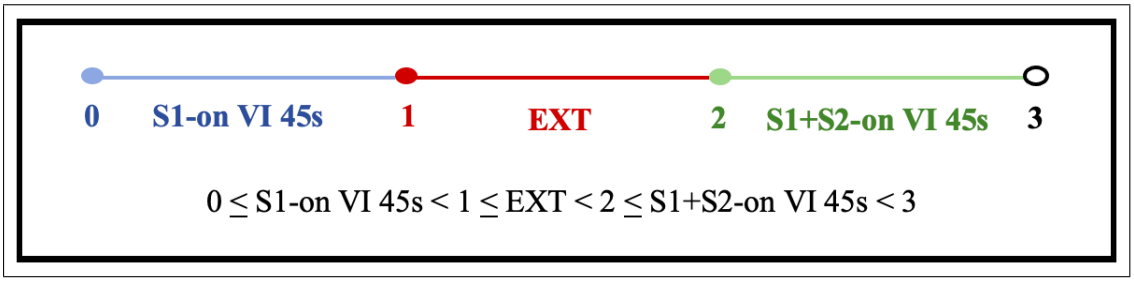

Figure 2.33: The three conditions in Compound Training [S1+S2: R →ed-DBS] and [S1+S2: R →no S1*] and their assigned ranges for randomly generated numbers by GS 4.

'S2: S1 on VI 45' employed the same schedule and the same stimulus as discrimination training [S1: R →S1* vs EXT] (Figure 2.34).

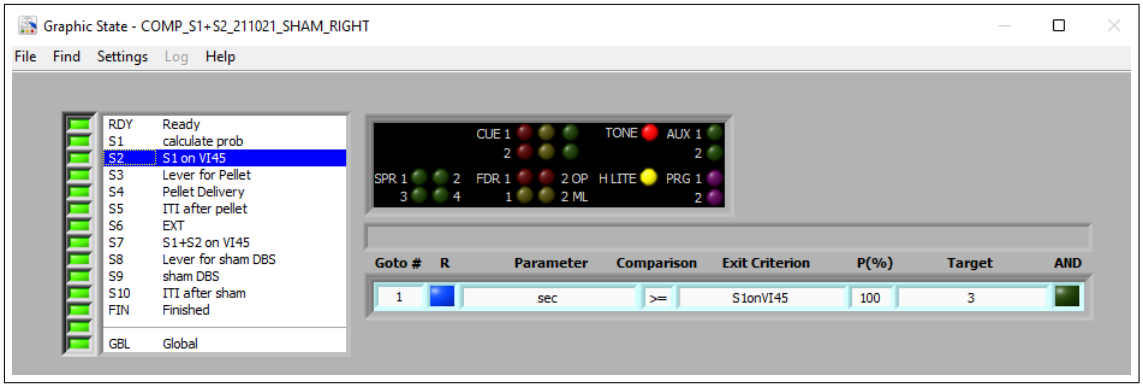

Figure 2.34: 'S2: S1 on VI 45' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

Following the end of randomly assigned variable time interval in this state, the protocol moved to 'S3: Lever for Pellet' (figure 2.35) and waited for the animal to press the lever once to deliver S1*, while the tone was continuously on. When the animal did not perform R during this 15s-long state, the protocol moved back to 'S1: calculate prob' to randomly pick and start another schedule.

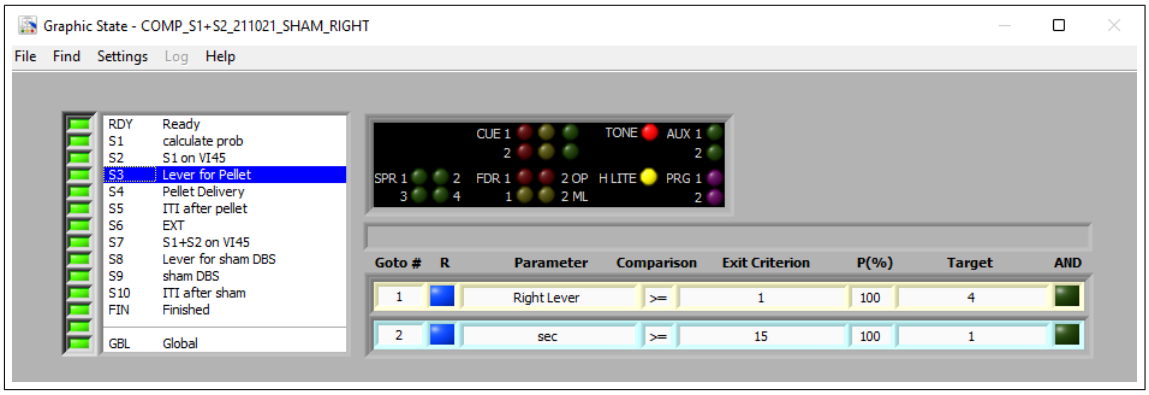

Figure 2.35: 'S3: Lever for Pellet' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

When the animal pressed the lever once during the 15-seconds wait period, the protocol delivered one S1* and jumped to 'S5: ITI after pellet' (Figure 2.36). In this state, the tone was still continuously on, but R did not result in S1* delivery.

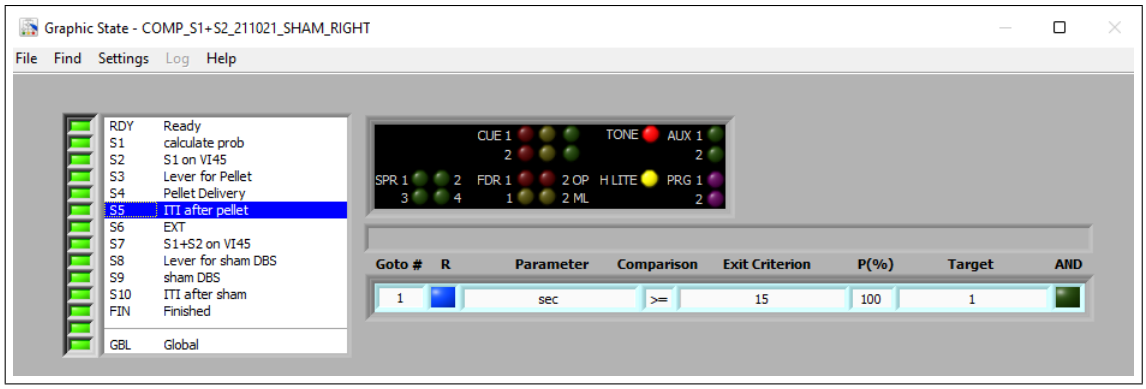

Figure 2.36: 'S5: ITI after pellet' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

'S6: EXT' (Figure 2.37) employed the same schedule as 'EXT' in discrimination training [S1: R →S1* vs EXT]. Following this state, the protocol went back to 'S1: calculate prob' to randomly pick and start another component.

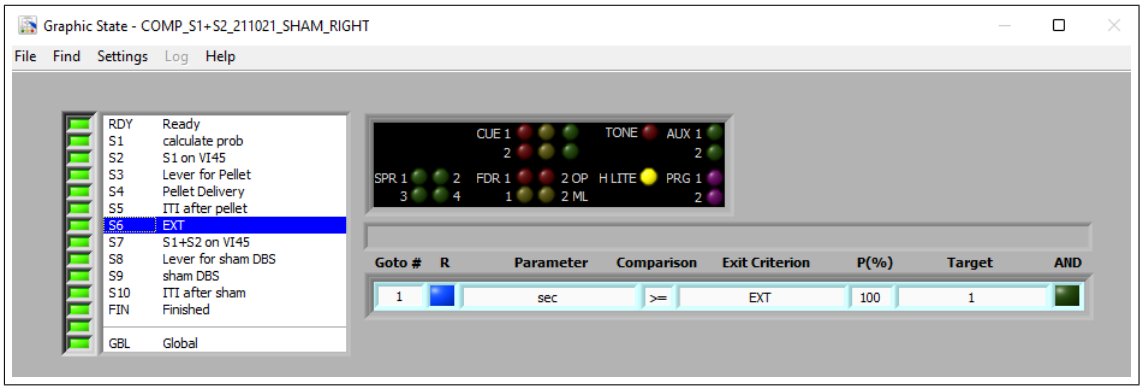

Figure 2.37: 'S6: EXT' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

'S7: S1+S2 on VI 45' (Figure 2.38) employed the same variable time interval schedule as 'S2: S1-on VI 45'. However, two stimuli, the tone and the triple cue light, were present conjointly in this state. Thus, related modules on the stimulus window were continuously on. As a random variable time interval ended, the protocol proceeded to 'S8: Lever for sham DBS'.

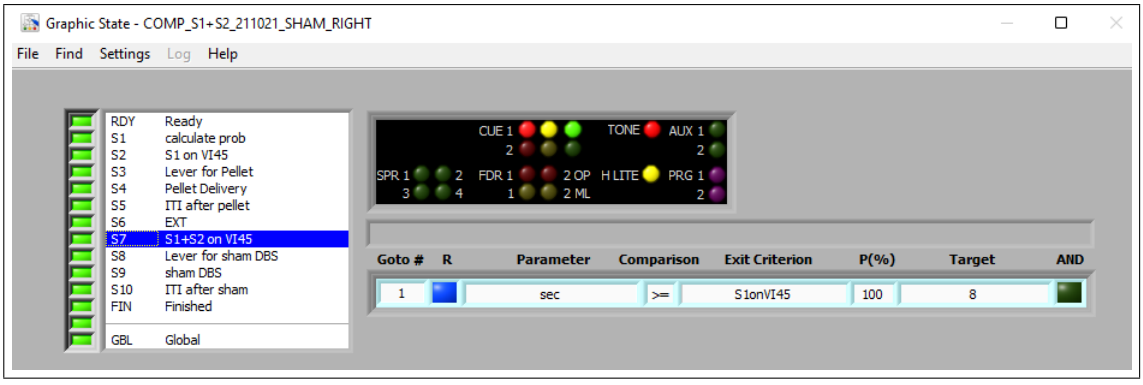

Figure 2.38: 'S7: S1+S2 on VI 45' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

Just like 'S3: Lever for Pellet', 'S8: Lever for sham DBS' waited for the animal's response for 15 seconds while the compound stimulus was continuously on (Figure 2.39). The protocol explained here was employed under compound training [S1+S2: R →no S1*] during the compound training of control animal #2. The protocol we employed for the experimental animal #4 also followed the same schedule, except that the state was named 'S8: Lever for DBS'. The control animal #2 was trained to press the right lever

while the experimental animal #4 was trained to press the left lever. Both animals went back to 'S1: calculate prob' when they did not perform R during the 15-second wait period. Following a lever press, #2 moved on to 'S9: sham DBS', in which he received no S1*, while #4 moved on to 'S9: DBS' (Figure 2.40).

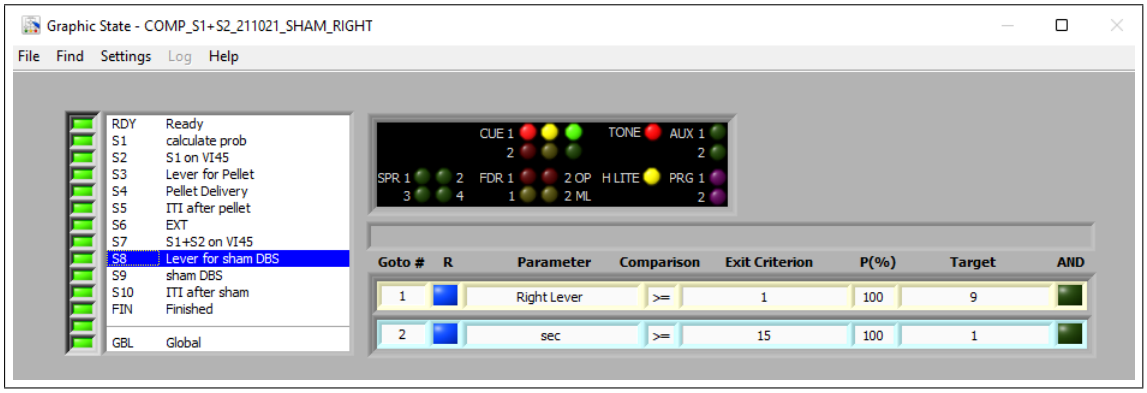

Figure 2.39: 'S8: Lever for sham DBS' state (no S1*) of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

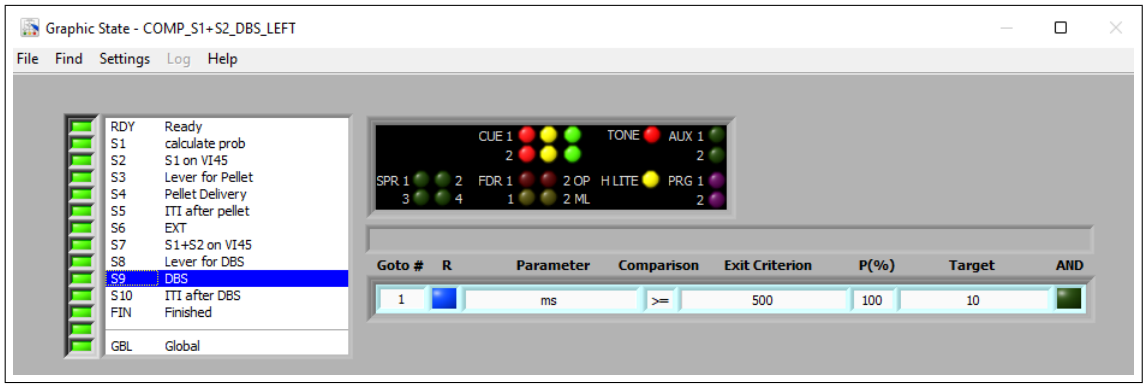

Figure 2.40: 'S9: DBS' state of Compound training [S1+S2: R →ed-DBS] protocol in Graphic State 4.

The control animal #2 was exposed to the same procedures in 'S9: sham DBS' as #4, except that the CUE 2 module in the stimulus window was not on, meaning that the current had not been turned on (Figure 2.41). Ideally, the control animal was supposed to be connected to the constant current stimulator via cable as well, however, his dental acrylic cap fell off during the first training session. As a result, #2 continued the remaining sessions without a cap.

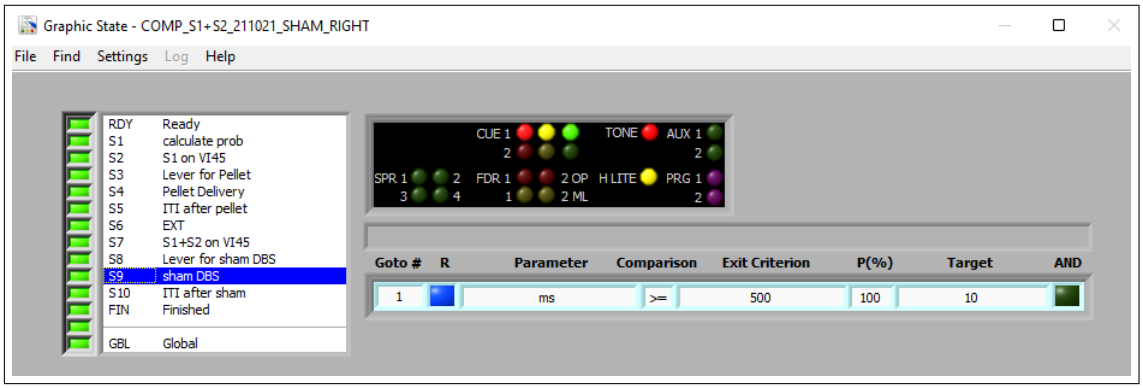

Figure 2.41: 'S9: sham DBS' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

Following no S1* and ed-DBS administration, the protocol switched to an ITI state. For #4, this state was named 'S10: ITI after DBS' but 'S10: ITI after sham' in the control animal training protocol. Both protocols involved the presentation of the compound stimulus (S1+S2: tone + triple cue light) for 15 seconds and then moved on to 'S1: calculate prob'. An example of this state from the control animal training protocol is shown in Figure 2.42.

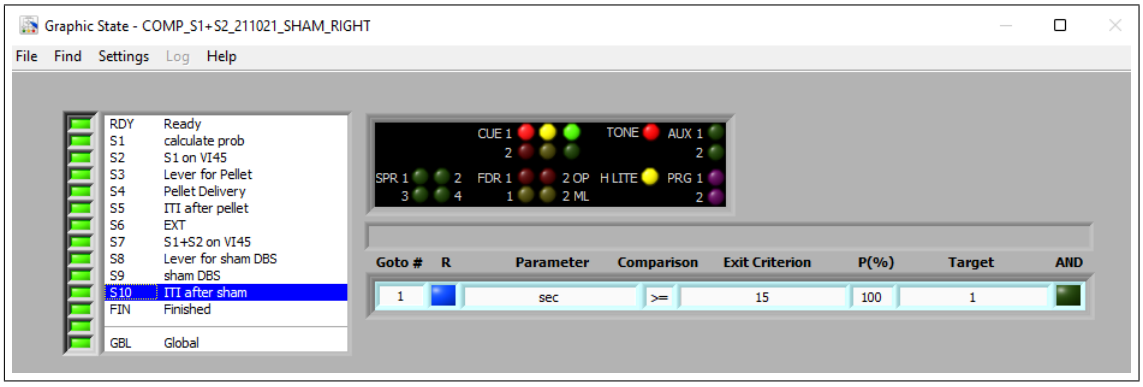

Figure 2.42: 'S10: ITI after sham' state of Compound training [S1+S2: R →no S1*] protocol in Graphic State 4.

2.14 Discrimination Training for S3: R →S1* vs EXT and Its Transcription in GS4

In this training, we used the same training protocol as discrimination training [S1: R →S1* vs EXT], except that we introduced the animal to a novel stimulus S3 (clicker) and used S3 clicker instead of S1 tone. Daily training sessions lasted 2 hours. Both animals (#2 and #4) ran this training for 7 sessions.

In discrimination training [S3: R →S1* vs EXT] the clicker was on during 'S1: S3 on VI45', 'S3: Lever Press', 'S4: Pellet Delivery', and 'S6: S3-on Interval' (Figure 2.43). These were the same states in which the tone was on in discrimination training [S1: R →S1* vs EXT] (Figure 2.21).

Figure 2.43: 'S1: S3 on VI45' state of Discrimination training [S3: R →S1* vs EXT] protocol in Graphic State 4.

Although the tone was continuous, the clicker, naturally, was not. We adjusted the clicker to click in pulses of 1 second ON and 2 seconds OFF periods (Figure 2.44). Pulses continued until the state was terminated.

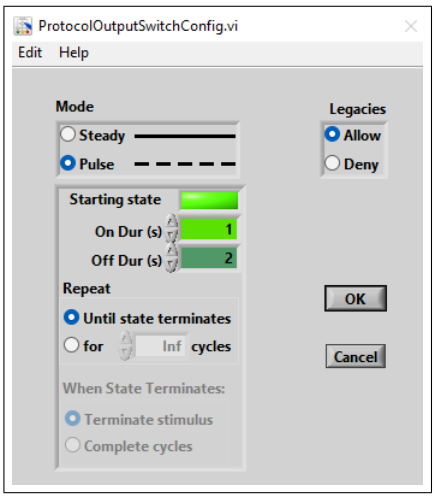

Figure 2.44: Clicker configuration window.

2.15 Summation Tests and Their Transcriptions in GS4

At the end of the discrimination training [S3: R →S1* vs EXT], we employed summation tests to test for conditioned inhibitory properties of the triple cue light S2. In the trainings preceding the summation tests, the animals had been exposed to the tone (S1), triple cue light (S2) together with the tone (S1+S2), and the clicker (S3). S1 and S3 each resulted in reinforcer (S1*) delivery when the animals fulfilled the S1* delivery criteria. Thus, we expected S1+S3 to gain excitatory value for the animals. As for S2, its value for the animal was expected to be defined by S2* (ed-DBS) in the case of #4 and by the absence of S1* (reinforcer) in the case of #2, assuming all other things are equal such as correct electrode placement.

In both summation test protocols, we employed 10 presentation blocks that each consisted of 4 60 s long components. The four components in Summation Test 1 consisted of 'S3-on VI 45s', 'S3+S2-on VI45s', and two OFF states that separated the first two and involved no stimulus presentation, similar to 'EXT' in the previous schedules. Each component lasted 60 s and was presented in random order. We assigned random orders of each component beforehand and created the protocol accordingly by using a random number generator. There was no S1* or S2* delivery during the presentation block schedules. We employed discrimination training [S3: R →S1* vs EXT] schedule

in between each presentation block to prevent extinction of R. In discrimination training [S3: R →S1* vs EXT], S1* was delivered as long as the criteria were met by the animal. One training session lasted until 10 presentation blocks and 9 discrimination training [S3: R →S1* vs EXT] were completed. Ahead of the first summation test, we also administered a short (45 min-long) S3-on VI45 session. The day after the first summation test, we trained the animals on [S3: R →S1*] for reacquisition of R. On day 3, we tested for excitatory properties of S3+S1 in Summation Test 2. The protocol was the same as Summation Test 1, except that the 4 components consisted of 'S3-on VI 45s', 'S3+S1-on VI 45s', 'S1-on VI 45s', and an OFF state.

We calculated the suppression ratio by dividing RR (Response Rate: R/min) during the compound CS (S3+S2 for Summation Test 1; S3+S1 for Summation Test 2) by the sum of all RR (sum of RR to S3+S2 and RR to S3 for Summation Test 1; sum of RR to S3+S1 and S3 as well as sum of RR to S3+S1 and S1 for Summation Test 2). As explained in detail in Hall et al. [35], we considered a value greater than 0.5 as an indicator of excitatory properties of the compound CS, and a value of less than 0.5 as an indicator of conditioned inhibition. 0.5 indicated no change.

A complete list of states in Summation Test 1 protocol is shown in Figure 2.45.

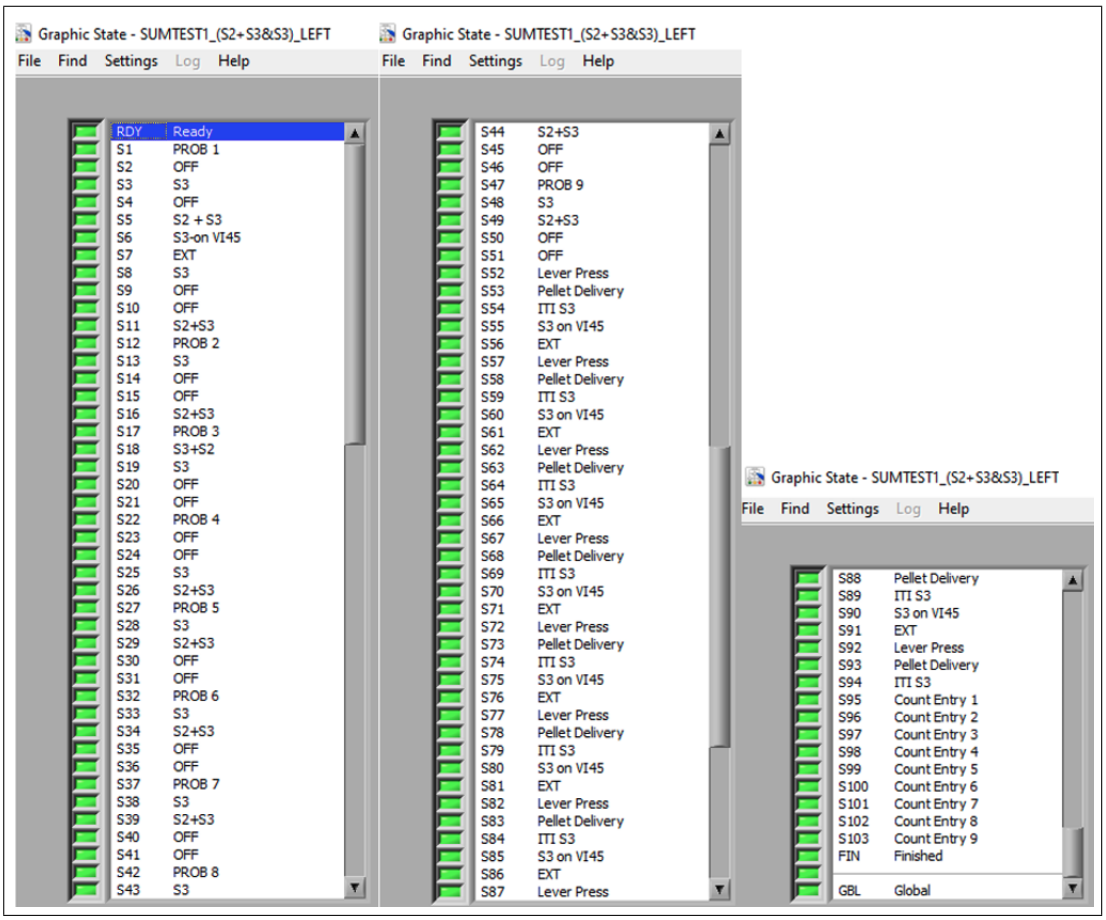

Figure 2.45: A complete list of states in Summation Test 1 protocol.

Following the 1 min 'RDY: Ready', the protocol moved to OFF state. The OFF state lasted 1 min like all components. During the state, there was no stimulus presentation, thus, no active module on the Stimulus Window. R did not result in S1* or S2* delivery (Figure 2.46).

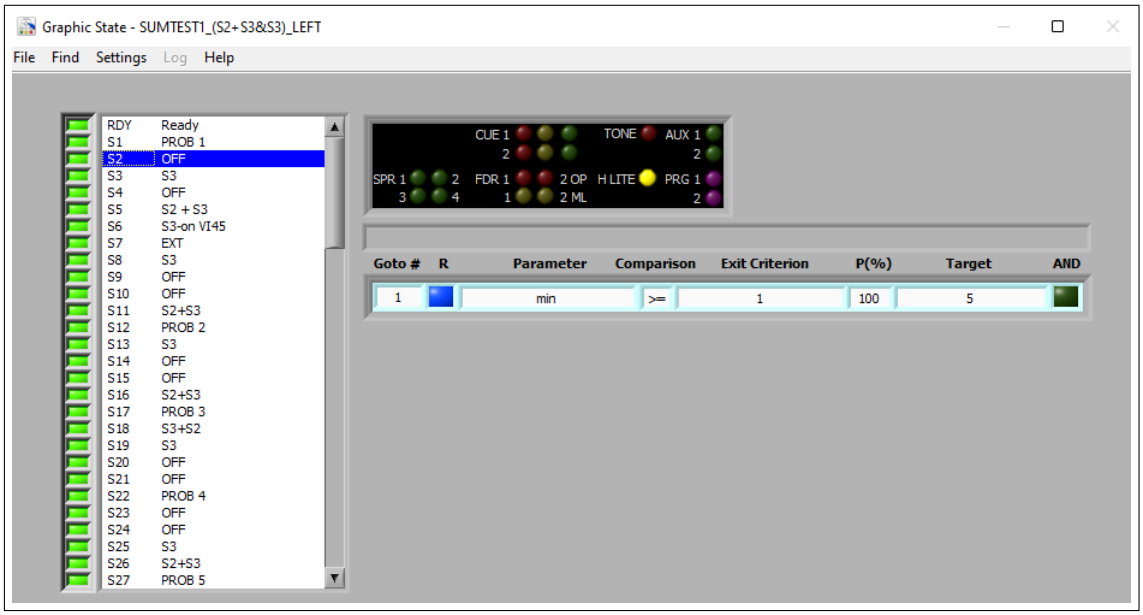

Figure 2.46: 'S2: OFF' state of Summation Test 1 protocol in Graphic State 4.

The OFF state was followed by a compound stimulus (S2+S3), that is, the triple cue and the clicker conjointly (Figure 2.47). In this state, the animal was exposed to stimulus S2 triple cue lights and stimulus S3 clicker conjointly. R did not result in S1* or S2* delivery. The state lasted 1 min and then switched to another OFF state.

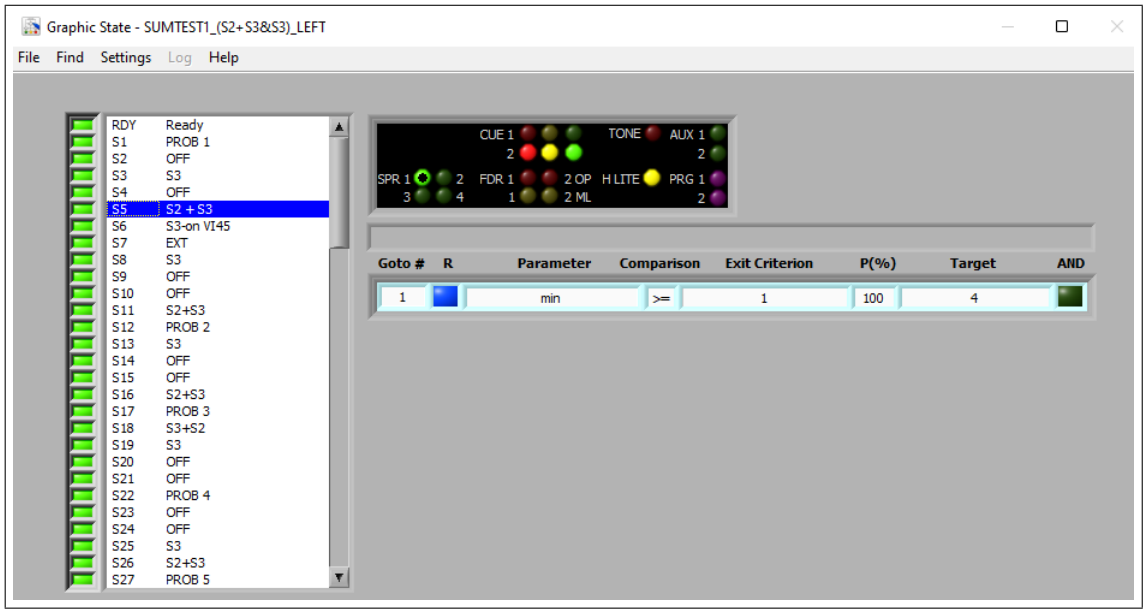

Figure 2.47: 'S5: S2 + S3' state of Summation Test 1 protocol in Graphic State 4.

Following this second OFF state, a S3 component came on, in which the animal was exposed to the S3 clicker for 1 min (Figure 2.48). After this, the protocol moved to 'S95: Count Entry 1'.

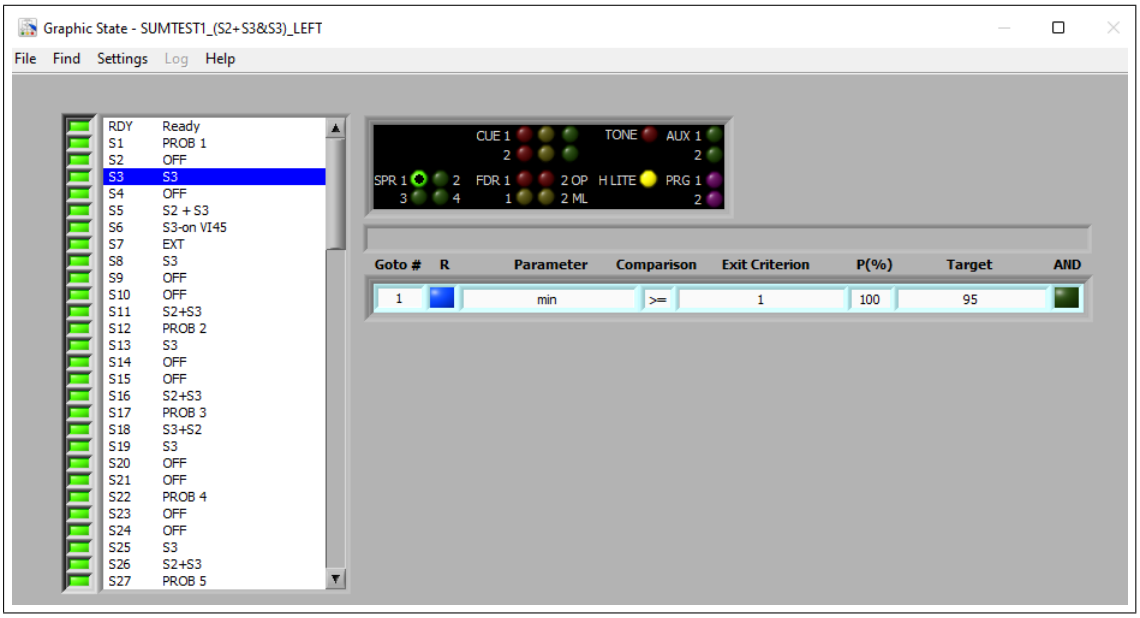

Figure 2.48: 'S3: S3' state of Summation Test 1 protocol in Graphic State 4.

Having completed one presentation block, the protocol entered a different block which consisted of 4 randomly selected components from the mult VI 45s/EXT schedule. In order to limit the number of components to four, we employed a "Count Entry" state which calculated the number of state entries and functioned as a counter (Figure 2.49). If the protocol entered this state less than 5 times, the protocol moved to 'PROB 1' which calculated the probability to either choose a VI45s component or EXT (Figure 2.50). There were 9 "Count Entry" states to calculate and limit the number of components to 4 following each presentation block.

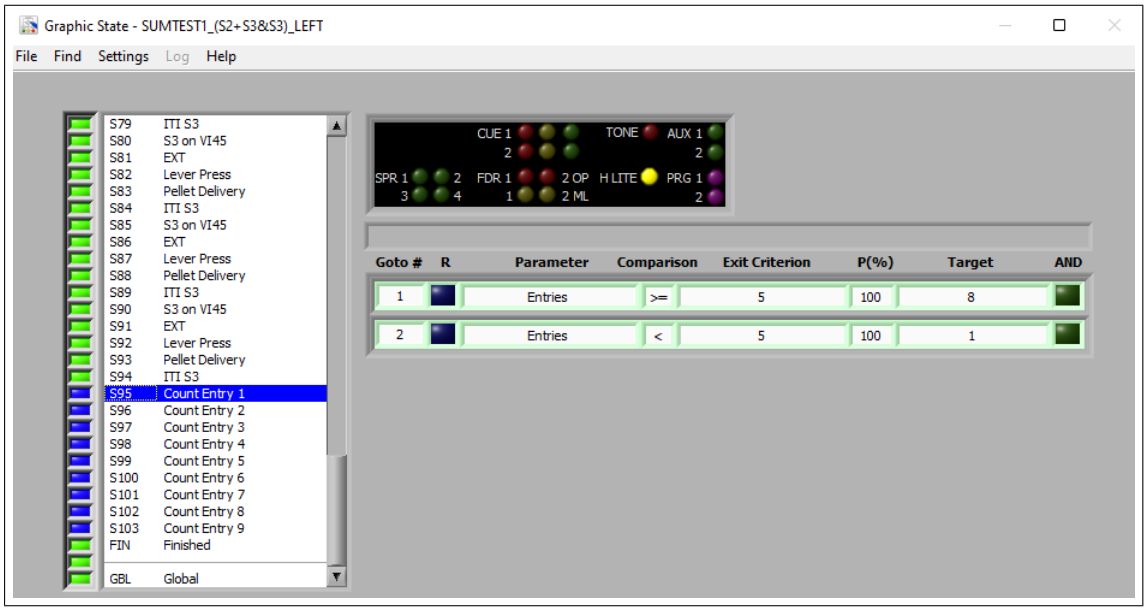

Figure 2.49: 'S95: Count Entry 1' state of Summation Test 1protocol in Graphic State 4.

In PROB 1 state, GS4 calculated the probability to either choose a VI45s component or EXT (Figure 2.50). This state followed the same principle as discrimination training [S1: R →S1* vs EXT] training protocol (Figure 2.26). Accordingly, the protocol either moved to 'S3-on VI 45' or to 'EXT'.

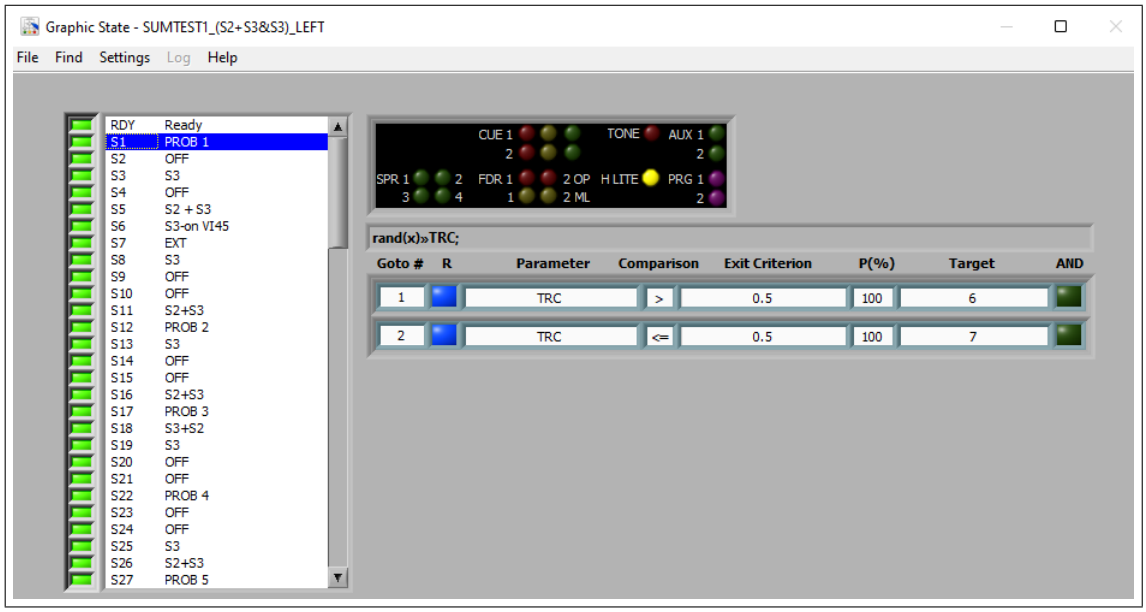

Figure 2.50: 'S1: PROB 1' state of Summation Test 1protocol in Graphic State 4.

When the protocol assigned 'S3-on VI 45' as the next state to be run, the protocol moved on to 'Lever Press', 'Pellet Delivery', 'ITI S3', respectively, as previously described for mult S3-on VI45s/EXT. If the animal did not press lever on time to collect a reward, the protocol jumped to S95: Count Entry 1' and either started a new presentation block or calculated the probability of S3-on VI 45s or EXT. A summary of the protocol flow is presented in Figure 2.51 below.

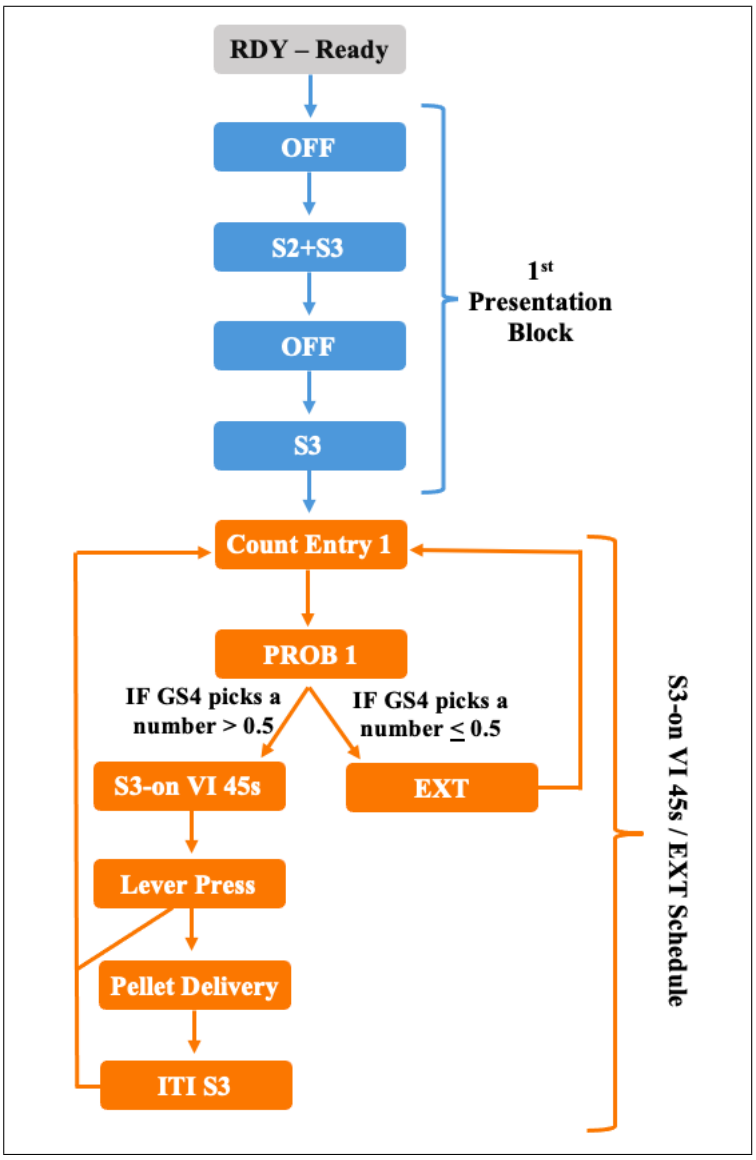

Figure 2.51: A presentation of protocol flow in Summation Test 1.

Summation Test 2 protocol was the same as Summation Test 1 protocol, except that the presentation blocks consisted of OFF, S1, S3, S1+S3 components. A representative block flow is shown in Figure 2.52 below.

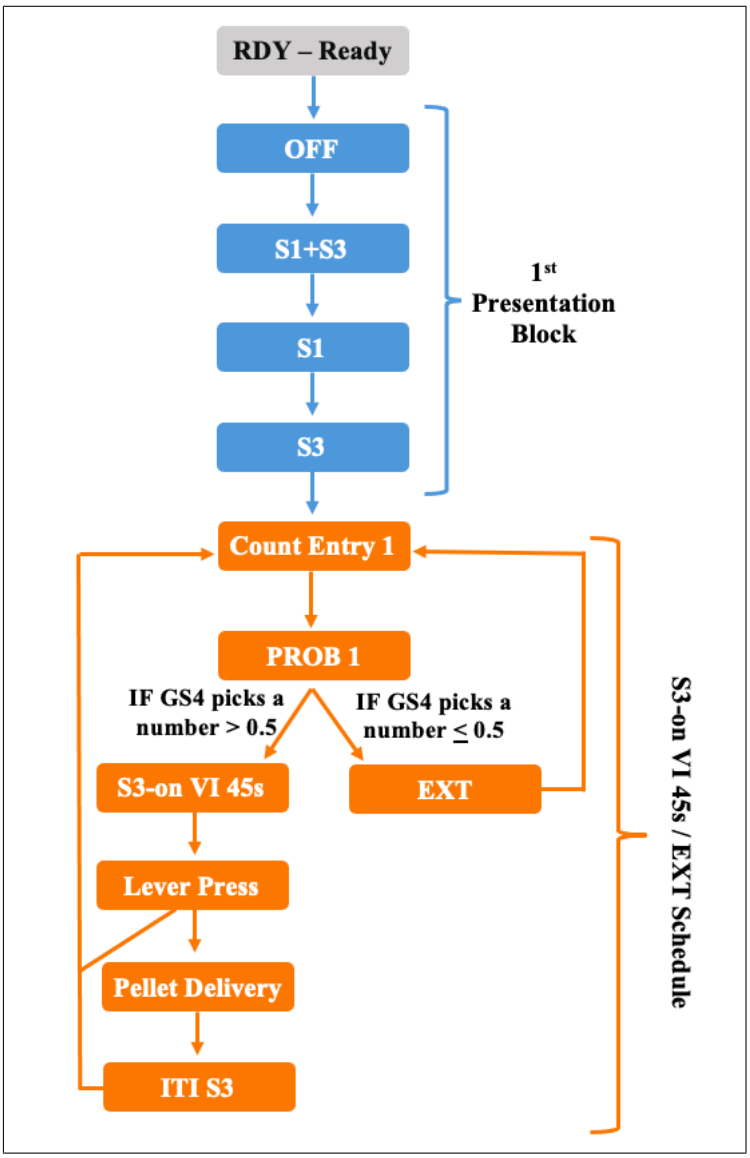

Figure 2.52: A presentation of protocol flow in Summation Test 2.

A complete list of states in Summation Test 2 protocol is shown in Figure 2.53.

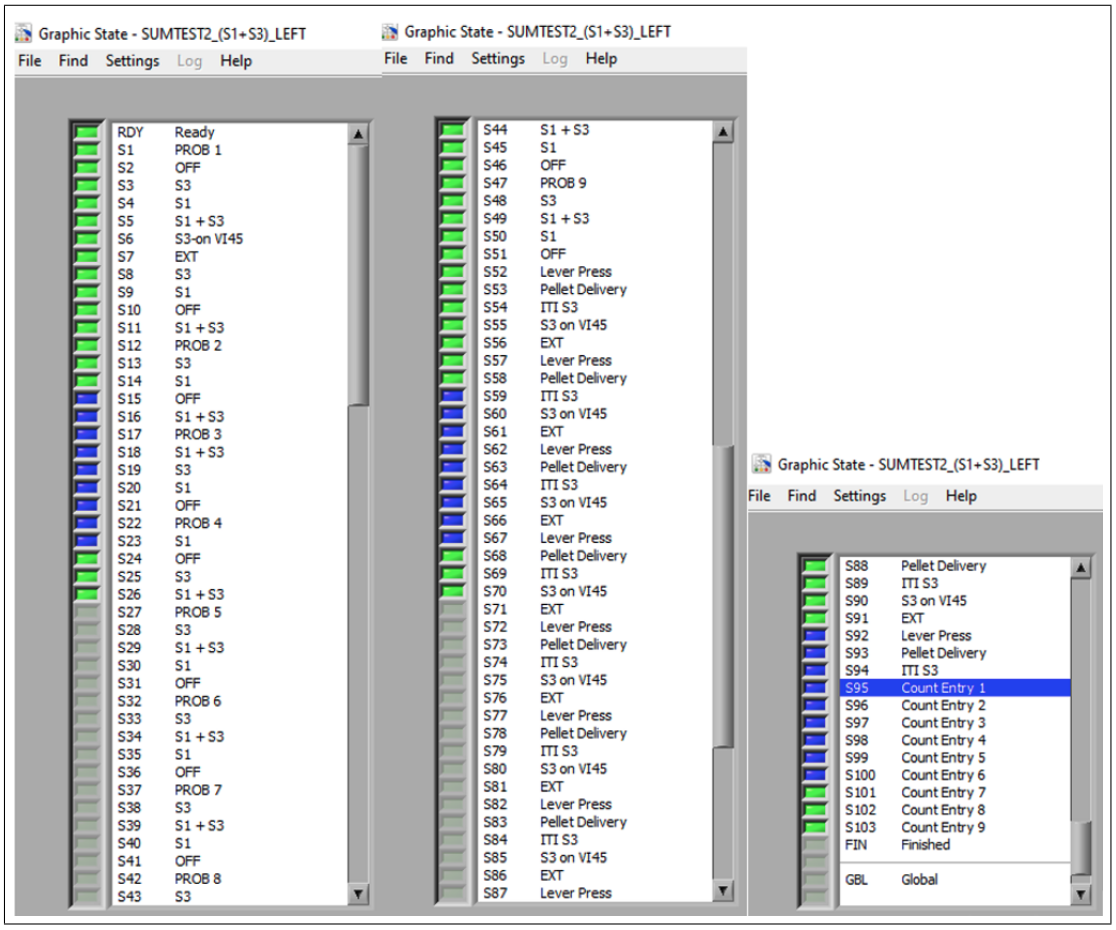

Figure 2.53: A complete list of states in Summation Test 2 protocol.

2.16 Apomorphine-Induced Rotation Test at the End of the Study

Both animals underwent the apomorphine-induced rotation tests one more time at the end of the study. #2 was intraperitoneally injected with low dose APO (0.05 mg/kg) and high dose APO (0.5 mg/kg). #4 was intraperitoneally injected with vehicle, low dose APO, and high dose APO.

2.17 Data Analysis

GS 4 creates data log sheets in a format that list the events in a single set of columns with each event labeled according to its type and data. We converted these data log sheets into Microsoft Excel sheets and exported them for further analyses. In addition to manual examination of Excel data sheets, we also utilized GS 4's Analysis Display, which is shown in Figure 2.54, to extract the following data from GS 4 for each session: number of entries to each state, number of pellets received by the animal, number of ed-DBS (or sham ed-DBS) received by the animal, and number of lever presses in each state ('S1 on VI45', 'S3 on VI45', 'S1+S2 on VI45', 'EXT').

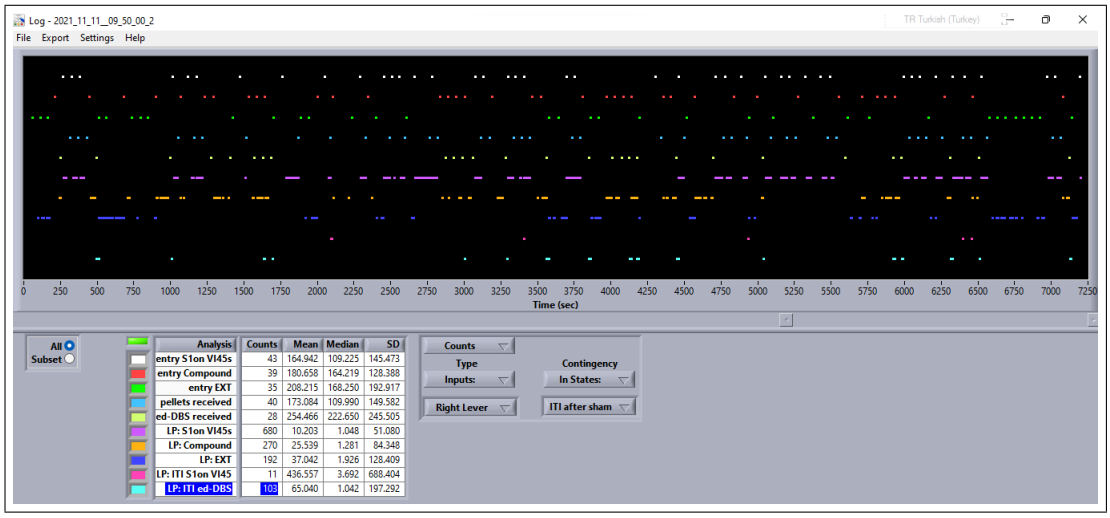

Figure 2.54: Analysis Display Window of GS 4, showing compound training [S1+S2: R →ed-DBS] scores of 2 in his last training session. The abbreviation "LP" stands for "Lever Press".

Since the occurrence of 'S1 on VI45', 'S3 on VI45', 'S1+S2 on VI45', and 'EXT' was random and was calculated by GS 4, the number of entries to each state changed from session to session. That is, daily number of entries to states were decided by GS 4 and were different in each session. Additionally, whenever the protocol entered a state (e. g. 'EXT'), the duration of the state was randomly chosen from a list of variable time intervals (e.g. 'EXT60'). Consequently, the animals were exposed to a various number of entries into and durations in each state, in every session. For instance, in the first session of discrimination training [S1: R →S1* vs EXT], 1 entered 'S1: VI45' 57

times (total duration: 42.25 mins) while he entered 'S2: EXT' 65 times (total duration: 61.36 mins). We evaluated the responding performance of animals measured by the "Response Rate" (RR: number of lever presses/min). RR was based on only the total duration in each state and did not take into account the number of entries into a state. We collected the total duration spent in a state at the end of a session which was shown by GS4 RunTime Display in seconds. Then, we divided this number by 60 to obtain the durations in minutes. Following the first batch of animals that were used in this thesis work, we adjusted the response rate calculation to evaluate the next batch's performance such that we took into account both the total duration in each state and the number of entries into a state. The resulting performance scores measured by both response rate calculations were similar, although not exactly the same.

We calculated the suppression ratio in Summation Test 1 by dividing RR in response to the compound stimulus (S3+S2) by the sum of RR in response to S3 and the compound. For the suppression ratio in Summation Test 2, we divided RR in response to the compound (S3+S1) by the sum of RR in response to S3 and the compound to calculate the suppression ratio for S3. We calculated the suppression ratio for S1 by dividing RR in response to the compound (S3+S1) by the sum of RR in response to S1 and the compound [35]. A value larger than 0.5 is considered excitatory, while a value smaller than 0.5 is considered inhibitory.

3. RESULTS

3.1 Instrumental Trainings

3.1.1 Magazine Training

After two 15-min magazine training sessions, the animals were able to pick up all the pellets from the magazine. Thus, we switched them to fixed ratio trainings.

3.1.2 Fixed Ratio (FR) Trainings

We found that lever pressing during Mult FR1-VT 20s training stabilized within three sessions. Therefore, the animals were shifted to the FR1 schedule. They required two sessions to continuously press the lever or collect 150 pellets per session (our criterion for successful completion of the schedule). They then proceeded to the FR 2 schedule and needed two sessions to continuously press the lever or collect 150 pellets per session before moving to the FR 5 schedule. The animals required one session to continuously press the lever or collect 150 pellets per session in both the FR 5 and FR 10 schedules. Next, they continued with the variable interval trainings.

3.1.3 Variable Interval (VI) Trainings

Lever pressing during VI 30s training stabilized within one session. Therefore, the animals were shifted to the VI 45s schedule. They required four sessions to continuously press the lever or collect 150 pellets per session.

Table 3.1 shows number of sessions the animals spent in each training protocol.

Table 3.1: Timeline for instrumental training schedules for all animals.

	Magazine Training	Mult FR 1-VT 20s	FR 1	FR 2	FR 5	FR 10	VI 30s	VI 45s
Number of Sessions	2	3	2	2	1	1	1	4

3.2 Discrimination Training for S1: R →S1* vs EXT

All animals reached the response rate criterion (a 5:1 ratio of responses during 'S1 on VI45' compared to 'EXT') in the second training session. The average response rates (number of lever presses/minute) during the components are shown in Table 3.2. RRs were calculated over 30 sessions for #1, 22 sessions for #2, 23 sessions for #3, and 21 sessions for #4.

Table 3.2: Average RRs for 'S1 on VI45' and 'EXT' components during discrimination training [S1: R →S1* vs EXT].

Animal ID	**#1**	**#2**	**#3**	**#4**
'S1 on VI45'	59.7	49.2	40.3	49.1
'EXT'	5.6	2.7	2.4	4.3

The cumulative response rates of the animals in 'S1 on VI 45' and 'EXT' components are presented in Figure 3.1.

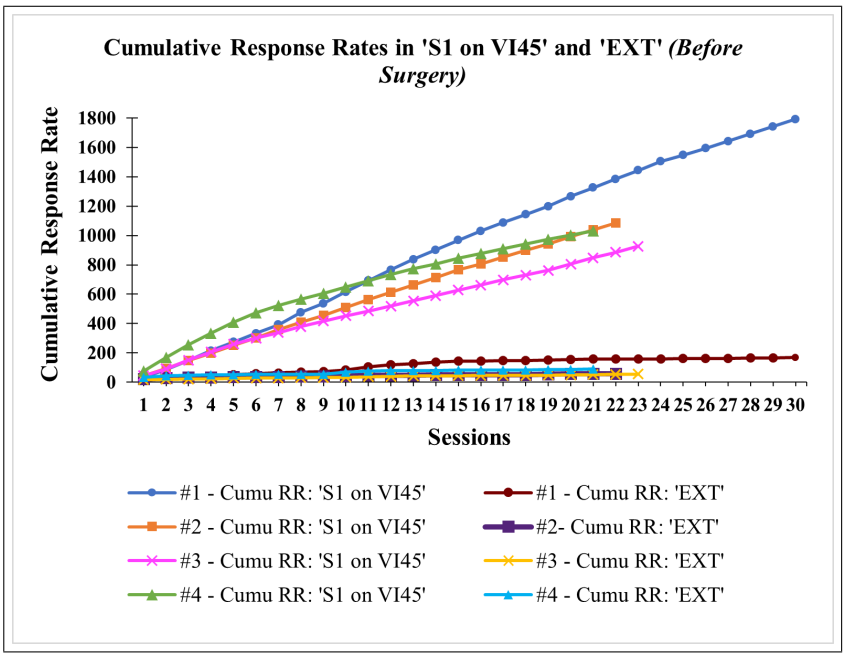

Figure 3.1: Cumulative RRs of animals in 'S1 on VI45' and 'EXT' states during discrimination training [S1: R →S1* vs EXT].

We found that all four animals acquired the discrimination. Figure 3.1 shows that the animals had high activity in 'S1 on VI45' state, which is indicated by a high number of lever presses, and low activity in 'EXT' state. #1 had the highest response rate among all animals in both states. #3 had the smallest response rate among all rats in 'S1 on VI45' state'. #4 had the highest response rate in 'S1 on VI45' state for the first 11 sessions when compared to the response rates of the other animals in the same state. Then, #4 started pressing the lever less and his cumulative response curve became flatter, possibly indicating that his performance became more efficient with training.

3.3 Hemiparkinson Rat Model Surgery and Implantation of Stimulation Electrode

2 of the 6 implanted animals (#4 and #2) recovered from the surgery and the remaining 4 died overnight. The 4 rats died following recovery from anesthesia. The

two animals that survived had only two supplementary anesthetic doses during surgery, in contrast to other animals that required supplementary injections more frequently.

Table 3.3: Surgery records (surgery date, the injection site, and status after surgery) of animals following surgeries.

Animal ID	**Surgery Date**	**Injection Site**	**Status After Surgery**
#6	August 5, 2021	Right MFB	Deceased
#5	August 9, 2021	Left MFB	Deceased
#4	August 14, 2021	Left MFB	Alive
#2	August 18, 2021	Right MFB	Alive
#3	August 19, 2021	Left MFB	Deceased
#1	September 3,2021	Right MFB	Deceased

After #6 died, we found excessive bleeding that had accumulated around his craniotomy site. After switching to hemostatic surgery sponge, we discovered less bleeding in/around the craniotomy site for the remaining surgeries.

3.4 Apomorphine-Induced Rotation Test

Following intraperitoneal injection with vehicle (spontaneous turn) #2 showed normal open field behavior. Moreover, neither the low dose of APO (0.05 mg/kg) nor the high dose (0.5 mg/kg) induced turning in #2. This was inconsistent with the intended manipulations (6-OHDA injection). #2 did not perform any turns in response to the additional 1 mg/kg APO dose.

#4 also showed normal open field behavior following intraperitoneal injection with vehicle. #4 turned once ipsilaterally to the lesion side (left MFB) upon his placement in the center of the arena. Following the low dose of APO injection (0.05 mg/kg), #4 exhibited two tight ipsiversive half turns, one immediately after another, upon placement in the center. Following the high dose of APO injection (0.5 mg/kg), #4 turned ipsiversive for the first time 2.5 minutes after his placement in the open field

arena. 30 seconds later, #4 showed repetitive tight ipsiversive full turns. During the first quarter of the session, #4 performed 203 tight successive ipsiversive turns. Figure 3.3 shows a capture during a tight ipsiversive full turn from the first quarter of the test session. #4 performed 143 tight ipsiversive turns during the second quarter and 38 tight ipsiversive turns during the third quarter. In the last quarter, the animal did not turn. There were no contraversive turns (contralateral to the lesion) during the whole session.

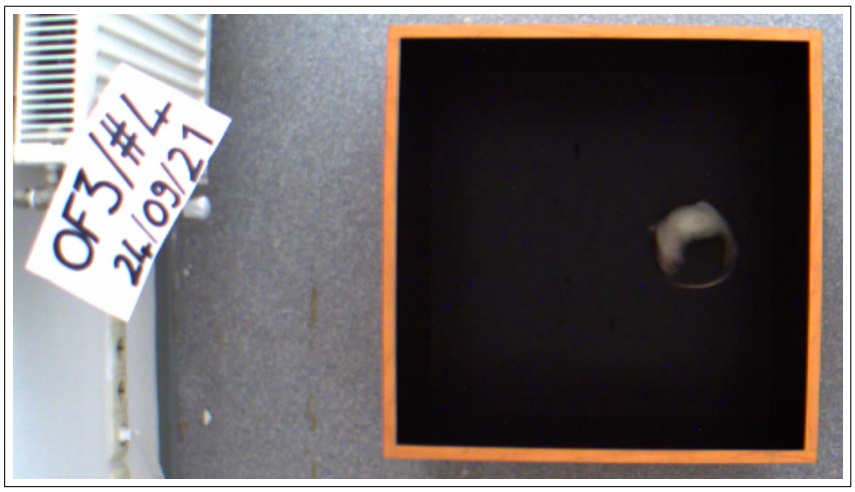

Figure 3.2: #4 performing a full tight ipsiversive turn during high-dose Apomorphine-induced rotation test session.

In conclusion, we found that #4 performed tight ipsiversive, although not contraversive, turns and continued the remaining procedures as an experimental animal. #2 proceeded the remaining procedures as a control animal.

3.5 Recovery Discrimination Training for S1: R →S1* vs EXT

After the surgery, #2 required 22 sessions of [S1: R →S1*] to recover to baseline performance whereas #4 needed 48 sessions. Table 3.4 shows the average RR of both animals in 'S1 on VI45' and 'EXT' before and after surgery. #2 was also trained under [S1: R →S1*] for 22 sessions before surgery while #4 was trained for 21 sessions.

Table 3.4: Average RR scores of #2 and #4 before and after surgery in 'S1 on VI 45' and 'EXT' components during discrimination training [S1: R →S1* vs EXT].

	'S1 on VI45'		**'EXT'**	
	Before Surgery	After Surgery	Before Surgery	After Surgery
#**2**(22 sessions)	49.2	38.9	2.7	10.5
#**4**(21 sessions)	49.1	40	4.3	26.4
#**4**(48 sessions)	NA	31.8	NA	14.6

Figure 3.3 shows that the cumulative response rates of #2 in both components were similar or close until session 7. Then, the cumulative response rate curve of 'EXT' state became flatter, showing no further increase in lever pressing rate for the remaining sessions. The cumulative response curves of #2 for both components show clear discrimination after session 7.

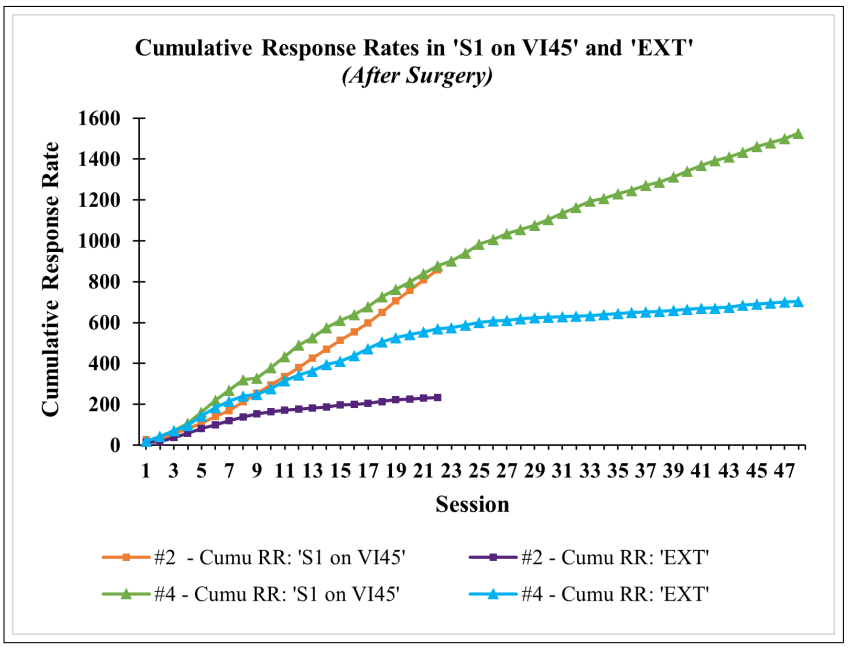

Figure 3.3: Cumulative RRs of #2 and #4 in 'S1 on VI45' and 'EXT' states during recovery discrimination training [S1: R →S1* vs EXT].

The cumulative response curve of #4 for 'EXT' flattens after session 21, which shows that he started pressing the lever less often with training. However, the number of lever presses in 'EXT' was not 5 times less than the number of lever presses in 'S1 on VI45', which indicates that the animal did not reach our criterion for discrimination.

Overall, we found that both animals performed more lever presses in 'EXT' after surgery compared to before (Figure 3.1 and Figure 3.3). However, the number of lever presses decreased with training for both animals. #2 showed successful discrimination before and after surgery whereas hPD rat #4 could not discriminate after surgery.

3.6 Compound Training for S1+S2: R →ed-DBS

Following recovery discrimination training [S1: R →S1* vs EXT], #2 underwent 22 sessions of compound training [S1+S2: R →no S1*] while #4 had 59 sessions of compound training [S1+S2: R →ed-DBS]. Out of 59 training sessions, 4 of them were excluded (sessions 38, 52, 53, 59) in data analyses due to tremors #4 experienced during the sessions, preventing him from performing. Table 3.5 shows the average RR scores of #2 (for a total of 22 sessions) and #4 (for the first 22 sessions and for a total of 55 sessions) for compound training [S1+S2: R →no S1*] and [S1+S2: R →ed-DBS], respectively. In compound training, #2 received sham ed-DBS (no S1*) and #4 received ed-DBS.

Table 3.5: Average RR scores of the animals for 'S1 on VI45', 'S1+S2 on VI45', and 'EXT' in compound training [S1+S2: R →ed-DBS] for #4 and [S1+S2: R →no S1*] for #2.

	#2	**#4**	
	22 sessions	**22 sessions**	**55 sessions**
'S1 on VI45'	24.9	22.3	24
'S1+S2 on VI45'	16.3	18.7	14.1
'EXT'	5.8	18.9	17.6

Figure 3.4 shows that #2 pressed the lever most often in 'S1 on VI45' state, followed by 'S1+S2 on VI45', and 'EXT'. The flattening of the curves indicate that the number of lever presses decreased with training for all three components. #4 preferred 'S1 on VI45' over 'EXT' and 'S1 on VI45', and 'EXT' over 'S1+S2 on VI45'. #4 performed similarly in 'S1+S2 on VI45' and 'EXT' states for the first 40 sessions. After that, he started pressing the lever less in 'S1+S2 on VI45' state. Although he performed similarly in 'S1 on VI45' to the other two states in the first 11 sessions, he increased the number of lever presses in this state over training.

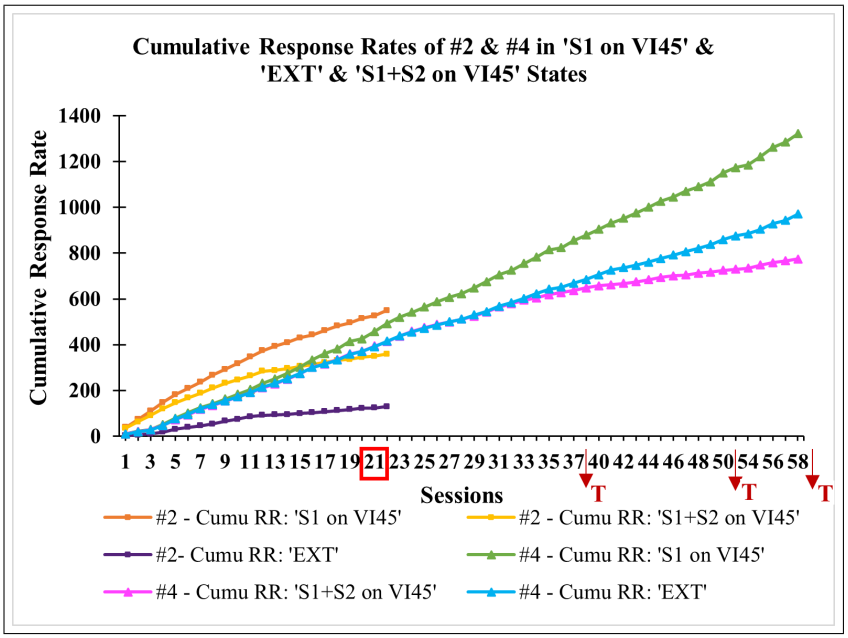

Figure 3.4: Cumulative response rates of #2 and #4 in 'S1 on VI45', 'EXT' and 'S1+S2 on VI45' components in compound training [S1+S2: R →ed-DBS] and [S1+S2: R →no S1*]. RRs were calculated over 22 sessions for #2 and 55 sessions for #4. Session 21 marks the date when #4 started receiving the remaining 34 sessions with the tone module completely uncovered. Red arrows and capital T's mark the intervals that correspond to the sessions during which #4 had tremors.

Figure 3.5 presents the number of omissions of the reinforcer (no S1*) in percent of the number of entries into the 'S1+S2 on VI45' state during the compound training for #2 [S1+S2: R→no S1*]. The orange bar represents average performance throughout the training. On average, #2 entered no S1* delivery state 77% of daily 'S1+S2' state entries. With training, the percentage dropped from 100% to under 40% (minimum).

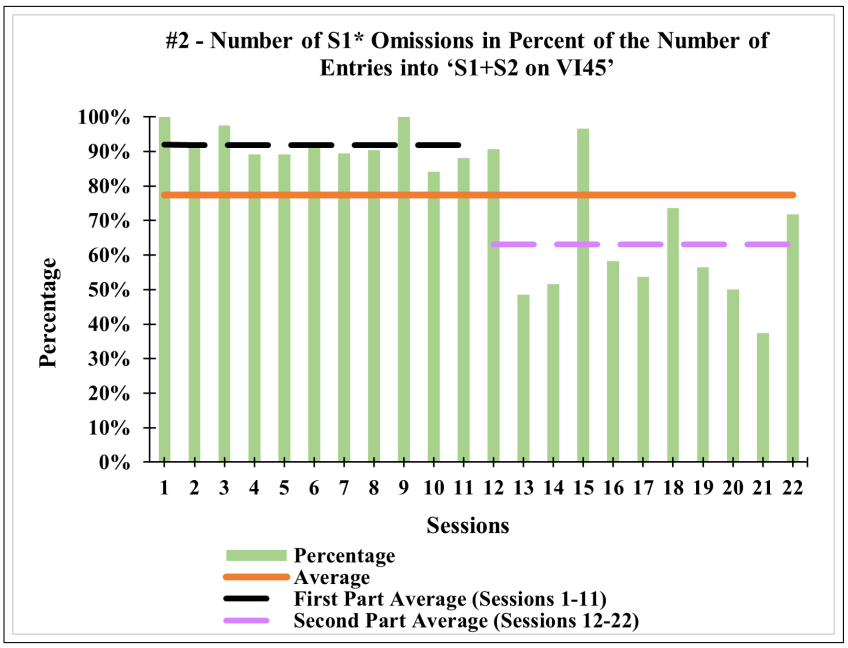

Figure 3.5: The number of daily S1* omissions in percent of daily number of 'S1+S2 on VI45' entries of #2 during the compound training [S1+S2: R→no S1*].

Figure 3.6 shows the number of times that ed-DBS was administered to #4 (in % of number of opportunities) during the compound training [S1+S2: R→ed-DBS]. On average, #4 received 69% of daily possible ed-DBS deliveries following entry into 'S1+S2' state. With training, the percentage dropped to 21% (minimum). Overall, we found that #4 had a greater relative decrease, which was 48% (from an average of 69% to a 21% minimum). The relative change for 2 was 39% (from an average of 77% to a 38% minimum).

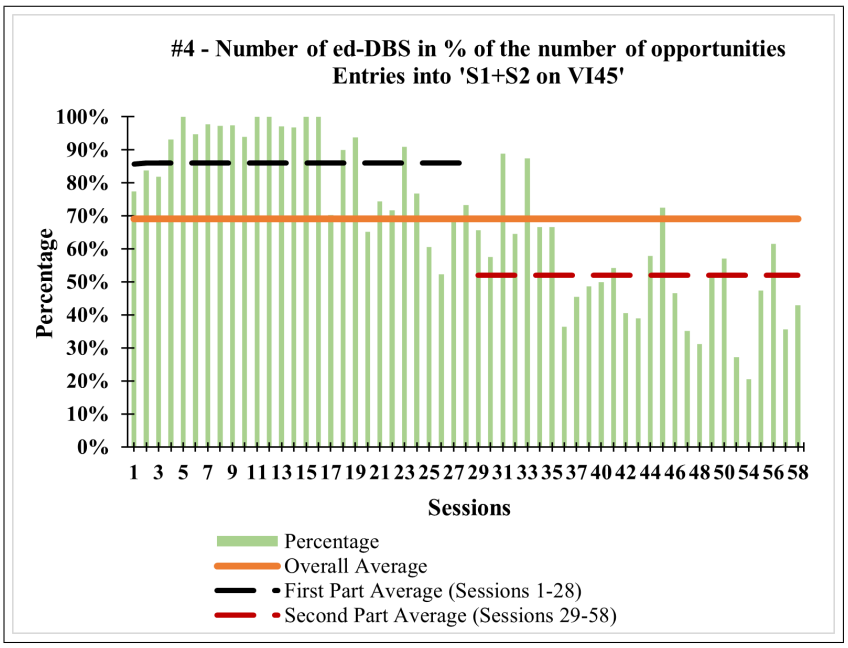

Figure 3.6: The number of ed-DBS (in % of the daily number of opportunities) entries into 'S1+S2 on VI45' of #4 during the compound training [S1+S2: R→ed-DBS].

3.7 Discrimination Training for S3: R→S1* vs EXT

Compound training [S1+S2: R→ed-DBS] or [S1+S2: R→no S1*] was followed by discrimination training [S3: R→S1* vs EXT] with S3 as the new stimulus (clicker) that predicted food (S1*). The compound randomly alternated with 'EXT', as before. Both rats (2 and 4) received 7 training sessions under this protocol. Their average RR scores across 7 training sessions are shown in Table 3.6.

Table 3.6: Average RR scores of the animals in 'S1 on VI45' and 'EXT' components in discrimination training [S3: R→S1* vs EXT].

	'S3 on VI45'	**'EXT'**
#2	27.2	4.5
#4	32.03	8.99

Figure 3.7 shows that both #2 and #4 increased the number of lever presses in 'S3 on VI45' state over training and discriminated between 'S3 on VI45' and 'EXT'.

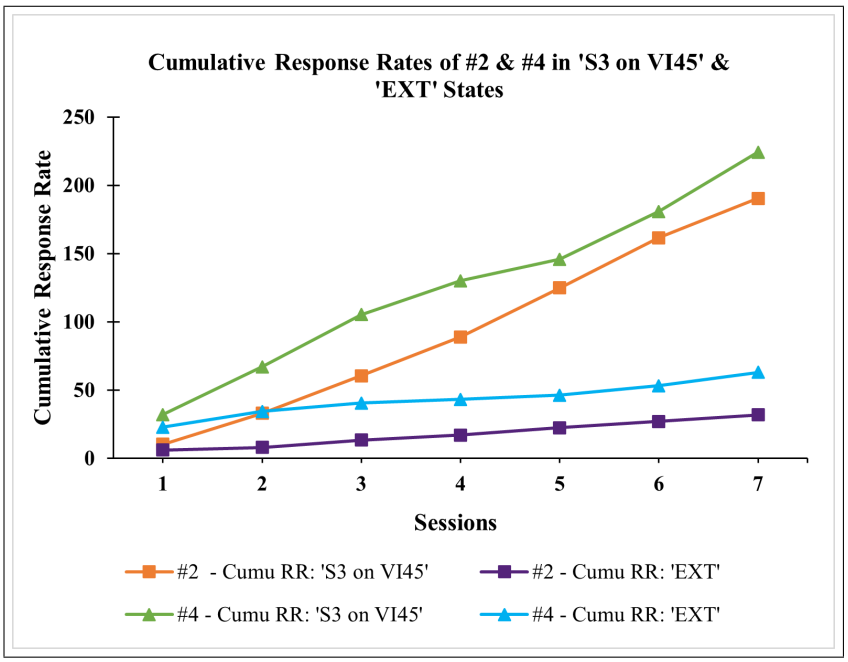

Figure 3.7: Cumulative RRs of #2 and #4 in 'S3 on VI45' and 'EXT' states during discrimination training [S3: R →S1* vs EXT].

Overall, #2 pressed the lever five times more in 'S3 on VI45' compared to 'EXT', starting in session 2 and reached the 5:1 criterion. #4 also reached the 5:1 criterion between session 3 and 5. He could not sustain the discrimination in the remaining sessions. However, his response rates for session 6 and 7 were close to the 5:1 criterion (RR for session 6: 4.98 lever press/min; RR for session 7: 4.43 lever press/min).

3.8 Summation Test 1

We found a suppression ratio of 0.29 (RR to compound divided by the sum of RR to S3 and RR to compound) for #2, and a ratio of 0.49 for #4. Figure 3.8 presents response rates of #2 and #4 in response to S3 alone and S2+S3 in compound.

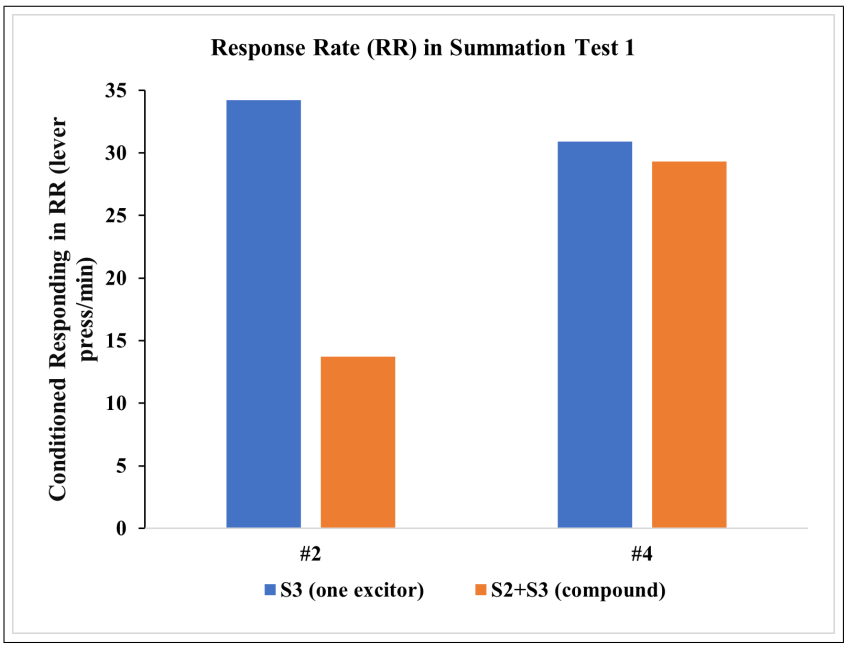

Figure 3.8: Response rates of #2 and #4 in Summation Test 1

3.9 Summation Test 2

Summation Test 2 had 3 components: S1+S3 (compound), S3 (clicker), and S1 (tone). For #2, the suppression ratio for the compound relative to the compound and S1 was 0.63 while the suppression ratio for the compound relative to the compound and S3 was 0.64. For #4, the suppression ratios were 0.7 and 0.64, respectively. Figure 3.9 shows the response rates of #2 and #4 in response to S1 alone, S3 alone, and S1+S3 in compound.

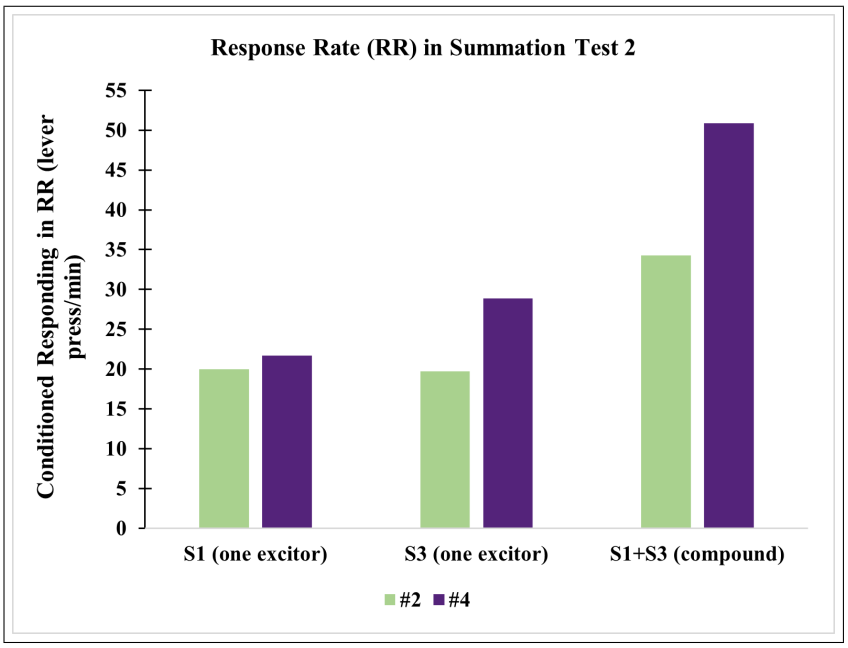

Figure 3.9: Response rates of #2 and #4 in Summation Test 2.

3.10 Apomorphine-Induced Rotation Test Scores at the End of the Study

#2 did not exhibit any turns (neither ipsiversive nor contraversive) following the low dose and high dose injections with APO (#2 did not receive injection with vehicle). #4 did not turn following injection with vehicle and the low dose injection with APO. Following the high dose (0.5 mg/kg APO), #4 displayed 275 tight ipsiversive turns during the first quarter. During the second quarter, he performed 276 tight ipsiversive turns. The third quarter was completed with 152 tight ipsiversive turns whereas the last quarter ended with 3 tight ipsiversive turns. There were no tight contraversive (contralateral to the lesion side: towards the right) turns during the session.

4. DISCUSSION

4.1 Discrimination Training for S1: R →S1* vs EXT

Our goal with discrimination training [S1: R →S1* vs EXT] was to train the animals to press the lever (R) to receive S1* when S1 was present but not when it was absent. We found that all animals learned the discrimination, consistent with other studies [26]. They pressed the lever more in 'S1 on VI45' than in 'EXT', and all animals reached our 5:1 criterion in which the response rate in 'S1 on VI45' was expected to be 5 times greater than the response rate in 'EXT'. All animals reached the criterion in the second session.

We also found differences in response pattern between animals. #1, #3, and #4 decreased the number of lever presses in both components over time while #2 had a constant response rate in 'S1 on VI45'. This difference in response patterns could be due to an inherent property of variable interval schedules. In variable interval schedules, the response rate can vary without affecting the reinforcement rate [36]. Therefore, it would be efficient for the animal to perform less R to receive the reinforcer as long as R is performed frequently enough to earn the reward. In summary, all animals discriminated between components and preserved the discrimination throughout sessions, regardless of their response patterns.

4.2 Hemiparkinson Rat Model and Apomorphine-Induced Rotation Test

In line with the literature [30], we unilaterally injected 12.5 µg 6-OHDA into the MFB to create a lesion that would be expected to result in at least 90% DA depletion in the damaged neostriatum. Such massive depletion is reflected in contraversive turning following DA challenge [29]. Of the two surviving rats following 6-OHDA injection,

only 4 showed rotational behavior that, in the literature, is related to DA receptor supersensitivity [29]. Literature [29] has shown that massive striatal DA depletion (>90%) typically leads to the development of a supersensitivity at postsynaptic DA neurons in the denervated neostriatum, resulting in deficits in extracellular DA activity. To compensate for these disturbances, the number of neostriatal D2-receptors can increase and thus lead to physiological supersensitivity [29]. The other major class of DA-receptors, D1-receptors, however, does not usually exhibit consistent modifications in affinity or binding [29]. Intake of APO, a DA agonist that can stimulate both D1 and D2 receptors, results in the induction of rotational behavior by acting on supersensitive postsynaptic DA receptors in the damaged neostriatum [29]. With reference to the literature, we aimed to evaluate the impact of APO on animals with massive unilateral 6-OHDA lesion. However, the direction of rotational behavior in #4 was not consistent with the literature [29]. It has been shown that unilateral 6-OHDA lesions of midbrain DA neurons can lead to a spontaneous turning asymmetry towards the lesion site (ipsiversive), depending on the neurotoxin dosage [29]. APO injection, on the other hand, induces contraversive turning (contralateral to the lesioned side) of animals, meaning that it reverses the turning asymmetry [29]. Upon injection, APO stimulates postsynaptic DA receptors in both hemispheres. However, DA receptor mediated supersensitivity on the lesioned hemisphere results in a higher DAergic impact in this hemisphere. As a result, asymmetry in rotational behavior by APO is caused due to higher stimulation of postsynaptic receptors on the lesioned side while the intact hemisphere goes through a normal stimulation [29].

Literature claims that rotational turning could be triggered with APO doses as low as 0.05 mg/kg [37],[38],[39]. Moreover, the same dose does not induce turning in intact rats [40]. Hence, we injected the animals with 0.05 mg/kg APO in the low dose test to see if the lesion was sufficient to induce rotational behavior with a low dose. Neither #4 nor #2 showed rotational behavior following the low dose of APO. These results differ from literature that has reported rotational behavior in response to 0.05 mg/kg APO-injection [29], [37],[38],[39]. Hence, it can be concluded that the lesions of #2 and #4 were not massive enough to induce rotational behavior in response to the 0.05 mg/kg APO injection.

Dose-dependent contraversive turning has been reported only after 0.5 mg/kg of APO in studies that administered a dose of 0.05, 0.25 or 0.5 mg/kg one day after 6-OHDA injection [29],[41],[42]. These studies have provided evidence that 0.05 and 0.25 mg/kg of APO do not induce rotational behavior one day after 6-OHDA lesion, yet 0.5 mg/kg dose of APO did. Accordingly, we injected the animals with 0.5 mg/kg (high dose) and found that this dose induced rotational behavior in #4, but not in #2. Additionally, #4 performed the majority of turns during the first half (first 30 min) of the test, which was also in line with the literature [29]. This performance is explained with the peak stimulation phase effect induced by APO [29]. One major difference from the literature, though, was that #4 performed ipsiversive and not contraversive turns. Possibly, the ipsiversive turn was caused by a faulty lesion which involved the contralateral hemisphere. Precise conclusion regarding the amount of lesion and its location is enabled by histological examinations, which is not in the scope of this project.

The dose of additional APO (1 mg/kg) given to #2 was determined in line with literature which provided evidence that APO doses as high as 1.5 mg/kg were safe to administer to rats [40]. That #2 did not show any rotational behavior in response to this dose either could be due to 1) an unsuccessful lesion, or 2) a lesion that was not massive enough to cause rotational behavior. In the latter case, reduced amounts of extracellular DA might have been compensated by the intact hemisphere.

In conclusion, #4 might have a hPD-like lesion but perhaps not in the right region or right hemisphere. Future evaluations involving histology will reveal the answer to this question.

4.3 Recovery Discrimination Training for S1: R →S1* vs EXT

Both #2 and #4 discriminated between 'S1 on VI45' and 'EXT' after surgery, yet it took a longer time compared to their pre-surgery performances. They also had lower RR scores in 'S1 on VI45' state and higher RR scores in 'EXT' state on average

after surgery compared to before. Therefore, we conclude that the surgical intervention (whether it creates a lesion or not) may negatively impact discrimination learning.

#4 increased the response rate in 'S1 on VI45' following the removal of tape that covered the tone module. By removing the cover, we increased the intensity of S1. Increasing the magnitude of S is known to increase stimulus salience and makes it more effective in conditioning [36]. As the tone became louder after session 21, the likelihood of improved attention also increased. Hence, the increase in salience likely supported the performance of #4.

Interestingly, we observed differences in the patterns of responding between the animals. One important difference was that RR of #4 decreased in both 'S1 on VI45' and 'EXT' over time while keeping the discrimination intact. This could indicate that #4 improved on efficiency over trials. On the other hand, #2 increased responding during 'S1 on VI45' and decreased responding during 'EXT'. Thus, we conclude that rats may show different patterns of discrimination learning.

4.4 Compound Training for S1+S2: R →ed-DBS and S1+S2: R →no S1*

S2 was presented to the animals for the first time with S1 conjointly to form a compound CS in compound training for #4 [S1+S2: R →ed-DBS] and for #2 [S1+S2: R →no S1*]. Hence, S2 had VS2 = 0 associative strength at the beginning of training [43]. S1, on the other hand, had already become an excitor for the animals during discrimination training [S1: R →S1* vs EXT] and recovery discrimination training [S1: R →S1* vs EXT].

During discrimination training [S1: R →S1* vs EXT], #2 had paired S1 and S1*, thus forming associative strength (V) between S1 (CS) and S1* (US). Then, in compound training [S1+S2: R →no S1*], S1+S2 signaled no S1* (no US) following

a lever press. According to the Rescorla-Wagner model, if a CS is first paired with a US (such as S1-S1* contingency established during discrimination training [S1: R →S1* vs EXT]), but then paired with no US when it is compounded with a novel CS (i.e. S2), the novel CS becomes an inhibitor [43],[44]. Consistent with this finding, we found that S2 negatively affected the response rate of #2, as reflected by a lower RR in 'S1+S2 on VI45' state (16.3 on average) compared to RR in 'S1 on VI45' state (24.9 on average). As a result, we conclude that S1 alone elicited lever press more frequently than the S1+S2 compound CS, due to inhibitory properties of S2.

Contrary to #2, #4 had a lower RR in 'S1+S2 on VI45' compared to 'EXT'. Overall, S2 gained inhibitory properties also for #4 and negatively affected the likelihood of responding when presented conjointly with S1. Yet, this inhibitory effect was stronger than 'EXT', which consisted of no stimulus presentation and no reinforcer delivery in response to behavior. EXT worked like a carry-over state; responding carried over from 'S1 on VI45' or the compound.

To understand the differences in behavior in response to 'S1 on VI45' and 'S1+S2 on VI45', it is crucial to state that S2 signaled different outcomes for #2 and #4 in compound training. For 2, S2 predicted the omission of food when presented with S1 conjointly. Although there was no S1* delivery in both 'S1+S2 on VI45' and 'EXT' in response to lever press, the former was different from the latter in that it also involved presentation of an excitatory stimulus (S1). Thus, the presence of S1 in 'S1+S2 on VI45' likely led to the expectation of S1*, which was then followed by frustration. In 'EXT' state, by contrast, there was no cue that predicted reinforcer delivery. What S2 predicted for #4 in compound training [S1+S2: R →ed-DBS] and why the animal responded less in 'S1+S2 on VI45' compared to 'S1 on VI45' could be argued in relation to the meaning of ed-DBS for the animal. If ed-DBS had no value for the brain, one could expect S2 to predict the absence of S1*, similar to a conditioned inhibitor, resulting in a reduced response rate. If ed-DBS was biologically meaningful and had excitatory properties, S2 would have become an excitor. As a result, RR in 'S1+S2 on VI45' could be similar to or higher than the RR in 'S1 on VI45'. Lastly, S2 could assume inhibitory properties if ed-DBS had an aversive effect on the animal. It seems

that S2 acquired inhibitory properties stronger than induced by 'EXT'. However, this is speculation since I was able to observe only one rat.

One should also note that it took much longer for #4 to learn the discrimination, compared to #2. Even then, it was quite slow. In addition to the effects of surgical intervention alone, the lesion, with impacts on rotational behavior shown in the APO-induced rotation test, might have negatively affected the ability of #4 to learn the discrimination [S1+S2: R →ed-DBS]. Based on the preliminary data from #2 and #4, we may conclude that the 6-OHDA lesion negatively impacted discrimination learning.

4.5 Discrimination Training for S3: R →S1* vs EXT

#2 discriminated between 'S3 on VI45' and 'EXT' after one training session and sustained his performance across the training sessions. 4, on the other hand, also reached the 5:1 criterion, but could not sustain his performance throughout the sessions. #4 showed worse performance than #2 in all three trainings post-surgery (recovery discrimination training for S1, compound training for S1+S2, and discrimination training for S3), but not before surgery. Therefore, we can argue that unilateral MFB lesion with 12.5 mu g 6-OHDA negatively affected discrimination learning in #4.

However, #2 and #4 pressed the lever more often in 'S3 on VI45' compared to 'EXT' and thus discriminated between the two components. These findings imply that S3 gained excitatory properties for the animals which predicted a US (S1*).

4.6 Summation Test 1

S3+S2 compound in Summation Test 1 had inhibitory properties over S3 for #2. This was expected given that the inhibitory stimulus S2 would suppress the excitatory properties of S3, in line with a conditioned inhibition effect as also shown by a study of

Hall et al. [35] and literature [36]. The suppression by S2 was lower for #4 than #2. In compound training [S1+S2: R →ed-DBS], the impact of S2 increased over time, as reflected by the decreasing response rate in 'S1+S2 on VI45'.

In conclusion, the compound training [S1+S2: R →ed-DBS] did not predict the performance of #4 in the Summation Test 1, whereas the performance of #2 in the Summation Test 1 was consistent with his performance in compound training [S1+S2: R →no S1*] and with literature [35],[36].

4.7 Summation Test 2

Reberg showed that two excitors, when presented together as a compound CS, produce a greater response compared to the response elicited by either of the excitors alone [45]. Several other studies supported Reberg's findings [26],[46],[47]. Both #2 and #4 increased their responses when they were presented with the compound (S1+S3) of two excitatory stimuli, S1 and S3. The suppression ratios for both stimuli were higher than 0.5, indicating that it was more excitatory compared to either S alone.

In conclusion, Summation Test 2 provided evidence that both animals established an association between S1 and S3 and S1* delivery, respectively.

5. LIMITATIONS AND SUGGESTIONS

In this study, we introduced the novel ed-DBS in which we tailored specific experiences of the subject to DBS administration. The goal of this study was to provide a proof-of-concept for our larger, BAP-funded project *Experience-driven (ed)-DBS to improve motor symptoms in the hemiparkinson rat model* (under grant number 15981). Since everything was built from scratch, we experienced a few limitations. First of all, we worked with a small sample size. We could only observe one hPD rat and one control rat. As the number of subjects accumulates over time, our BAP-funded project will deliver more confident observations to optimize our intervention protocols that involve ed-DBS administration. This is also true regarding our hemiparkinson rat model surgery protocol. Survival rate following surgery during this study was quite low (2/6). Further optimizations with a bigger sample size will yield more precise outcomes. Lastly, this study lacked histology. As a result, we were not able to verify 6-OHDA lesions histologically. Due to this major limitation, we could not answer some of our questions, such as the accuracy of lesion site.

When all the necessary steps are established, our BAP-funded project will be able to provide insights into the functions of ed-DBS. Outcomes of this study may help improve clinical applications of traditional DBS treatment.

REFERENCES

1. Middleton, Frank A., and Peter L. Strick, "Basal ganglia output and cognition: evidence from anatomical, behavioral, and clinical studies," *Brain and Cognition*, 2000.

2. Monchi, O., Petrides M., Doyon J., Postuma, R. B. et al., "Neural bases of set-shifting deficits in Parkinson's disease," *Journal of Neuroscience*, 2004.

3. Rodriguez-Oroz, M. C., Jahanshahi, M., Krack, P., Litvan, I. et al., "Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms," *The Lancet Neurology*, 2009.

4. Cronin-Golomb, A., Reynolds, G. O., Salazar, R. D., and Saint-Hilaire, M.-H., "Parkinson's disease and Parkinson-plus syndromes," in M. L. Alosco & R. A. Stern (Eds.), The Oxford Handbook of Adult Cognitive Disorders (pp. 600-630). Oxford University Press, 2019.

5. Parkinson, James, "An essay on the shaking palsy," *The Journal of Neuropsychiatry and Clinical Neurosciences*, 2002.

6. Saeed, U., Compagnone, J., Aviv, R. I., Strafella, A. P. et al., "Imaging biomarkers in Parkinson's disease and Parkinsonian syndromes: current and emerging concepts," *Translational Neurodegeneration*, 2017.

7. Lozano, Christopher S., Joseph Tam, and Andres M. Lozano., "The changing landscape of surgery for Parkinson's Disease," *Movement Disorders*, 2018.

8. Tröster, Alexander I., "Some clinically useful information that neuropsychology provides patients, carepartners, neurologists, and neurosurgeons about deep brain stimulation for Parkinson's disease," *Archives of Clinical Neuropsychology*, 2017.

9. Benabid, Alim Louis., "Deep brain stimulation for Parkinson's disease," *Current Opinion in Neurobiology*, 2003.

10. Ramirez-Zamora, Adolfo, and Jill L. Ostrem., "Globus pallidus interna or subthalamic nucleus deep brain stimulation for Parkinson disease: a review," *JAMA Neurology*, 2018.

11. Bronstein, J. M., Tagliati, M., Alterman, R. L., Lozano, A. M. et al., "Deep brain stimulation for Parkinson disease: an expert consensus and review of key issues," *Archives of Neurology* 68.2, 2011.

12. Ferrini, A., Steel, D., Barwick, K. and Kurian, M.A., "An update on the phenotype, genotype and neurobiology of ADCY5-related disease," *Movement Disorders*, 2021.

13. Gerfen, Charles R., "The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia," *Annual Review of Neuroscience*, 1992.

14. Bruet, N., Windels, F., Bertrand, A., Feuerstein, C. et al., "High frequency stimulation of the subthalamic nucleus increases the extracellular contents of striatal dopamine in normal and partially dopaminergic denervated rats," *Journal of Neuropathology & Experimental Neurology*, 2001.

15. Pazo, J.H., Höcht, C., Barcelo, A.C., Fillipini, B. et al., "Effect of electrical and chemical stimulation of the subthalamic nucleus on the release of striatal dopamine," *Synapse*, 2010.

16. Huotarinen, A., Penttinen, A-M., Back, S., Voutilainen, M.H. et al., "Combination of CDNF and deep brain stimulation decreases neurological deficits in late-stage model of Parkinson's disease," *Neuroscience*, 2018.

17. Deuschl, G., Schade-Brittinger, C., Krack, P., Volkmann, J. et al., "A randomized trial of deep-brain stimulation for Parkinson's disease," *New England Journal of Medicine*, 2006.

18. Williams, A., Gill, S., Varma, T., Jenkinson, C. et al., Williams, Adrian, et al. "Deep brain stimulation plus best medical therapy versus best medical therapy alone for advanced Parkinson's disease (PD SURG trial): a randomised, open-label trial," *The Lancet Neurology*, 2010.

19. Okun, M.S., Gallo, B.V., Mandybur, G., Jagid, J. et al., "Subthalamic deep brain stimulation with a constant-current device in Parkinson's disease: an open-label randomised controlled trial," *The Lancet Neurology*, 2012.

20. Nickl, R.C., Reich, M.M., Pozzi, N.G., Fricke, P. et al., "Rescuing suboptimal outcomes of subthalamic deep brain stimulation in Parkinson disease by surgical lead revision," *Neurosurgery*, 2019.

21. Koeglsperger, T., Palleis, C., Hell, F., Mehrkens, J. et al., "Deep brain stimulation programming for movement disorders: current concepts and evidence-based strategies," *Frontiers in Neurology*, 2019.

22. Schultz, W., "Multiple dopamine functions at different time courses," *Annual Review of Neuroscience*, 2007.

23. Schultz, W., "Predictive reward signal of dopamine neurons," *Journal of Neurophysiology*, 1998.

24. Schultz, W., "Dopamine signals for reward value and risk: basic and recent data," *Behavioral and Brain Functions*, 2010.

25. Albayrak, E. B., "Generating non-normal distributions for interval schedules: effects on instrumental behavior," Master's thesis, Bogazici University, Istanbul, Turkey, 2023.

26. Lombas, A.S., Kearns, D.N., and Weiss, S.J., "A comparison of the effects of discriminative and Pavlovian inhibitors and excitors on instrumental responding," *Behavioral Processes*, 2008.

27. Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, London: Elsevier, 6th ed., 2007.

28. Ostock, C.Y., Lindenbach, D., Goldenberg, A.A., Kampton, E. et al., "Effects of noradrenergic denervation by anti-DBH-saporin on behavioral responsivity to L-DOPA in the hemi-parkinsonian rat," *Behavioural Brain Research*, 2014.

29. Schwarting, R.K.W. and Huston, J.P., "The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments," *Progress in Neurobiology*, 1996.

30. Petri, D., Pum, M., Vesper, J., Huston, J.P. et al., "GABAA-receptor activation in the subthalamic nucleus compensates behavioral asymmetries in the hemiparkinsonian rat," *Behavioural Brain Research*, 2013.

31. Darbaky, Y., Forni, C., Amalric, M. and Baunez, C., "High frequency stimulation of the subthalamic nucleus has beneficial antiparkinsonian effects on motor functions in rats, but less efficiency in a choice reaction time task," *European Journal of Neuroscience*, 2003.

32. Rouaud, T., Lardeux, S., Panayotis, N., Paleressompoulle, D. et al., "Reducing the desire for cocaine with subthalamic nucleus deep brain stimulation," *Proceedings of the National Academy of Sciences*, 2010.

33. Huotarinen, A., Penttinen, A.M., Bäck, S., Voutilainen, M.H. et al., "Combination of CDNF and deep brain stimulation decreases neurological deficits in late-stage model Parkinson's disease," *Neuroscience*, 2018.

34. Pavon, N., Martin, A. B., Mendialdua, A., and Moratalla, R., "ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice," *Biological Psychiatry*, 2006.

35. Hall, G., Prados, J. and Sansa, J., "Modulation of the effective salience of a stimulus by direct and associative activation of its representation," *Journal of Experimental Psychology: Animal Behavior Processes*, 2005.

36. Bouton, E., M., *Learning and Behavior: A Contemporary Synthesis*, Sinauer Associates, 2007.

37. Cadet, J.L., Brannock, C., Krasnova, I.N., Ladenheim, et al., "Methamphetamine-induced dopamine-independent alterations in striatal gene expression in the 6-hydroxydopamine hemiparkinsonian rats," *PLoS One*, 2010.

38. Campbell, J.C., Jeyamohan, S.B., De La Cruz, P., Chen, N., et al. "Place conditioning to apomorphine in rat models of Parkinson's disease: Differences by dose and side-effect expression," *Behavioural Brain Research*, 2014.

39. Björklund, A. and Dunnett, S.B., "The amphetamine induced rotation test: a reassessment of its use as a tool to monitor motor impairment and functional recovery in rodent models of Parkinson's disease," *Journal of Parkinson's Disease*, 2019.

40. Konitsiotis, S., Kafetzopoulos, E., Anastasopoulos, D. and Blanchet, P.J., "Opposite rotation induced by dopamine agonists in rats with unilateral lesions of the globus pallidus or substantia nigra," *Behavioural Brain Research*, 1998.

41. Oberlander, C., Euvrard, C., Dumont, C. and Boissier, J.R., "Circling behaviour induced by dopamine releasers and/or uptake inhibitors during degeneration of the nigrostriatal pathway," *European Journal of Pharmacology*, 1979.

42. Labandeira-Garcia, J.L., Rozas, G., Lopez-Martin, E., Liste, I., et al., "Time course of striatal changes induced by 6-hydroxydopamine lesion of the nigrostriatal pathway, as studied by combined evaluation of rotational behaviour and striatal Fos expression," *Experimental Brain Research*, 1996.

43. Rescorla, R. A. and Wagner, A.R., "A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement," *Current Research and Theory*, 1972.

44. Rescorla, R. A., "Summation and retardation tests of latent inhibition," *Journal of Comparative and Physiological Psychology*, 1971.

45. Reberg, D., "Compound tests for excitation in early acquisition and after prolonged extinction of conditioned suppression" *Learning and Motivation*, 1972.

46. Weiss, S.J., "Attentional processes along a composite stimulus continuum during free-operant summation" *Journal of Experimental Psychology*, 1969.

47. Weiss, S.J., "Discrimination training and stimulus compounding: consideration of non-reinforcement and response differentiation consequences of SΔ," *Journal of the Experimental Analysis of Behavior*, 1971.