

Assessment of nose-poking responses in mice mediated by a neural stimulation system

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ABSTRACT

In research, evaluating the behavioral response of animals by training certain behaviors is crucial for assessing the response of neurons to stimulation signals. For animal behavioral activities, there are many approaches to research and evaluation. Thus, the research process needs to understand the stimulation factors that contribute to the behavioral response. The research involves formulating algorithms as the basis for building tests to understand the characteristics of the research subject and for building boundary conditions of the problem. The study of central nervous system activity at molecular, subcellular, neuron or neural network levels is one of the key directions. However, in Vietnam, there is no specialized center to assess the physiological activities of the nervous system and the electrical stimulation and recording system of neurons, which is a new research direction nationally.

Keywords: Electrical stimulation; Neurons; Stimulation signals; Behavioral response.

1. INTRODUCTION

In medical and pharmacological research, rewards play an important role in research models to assess animals' learning, speed, and ability in memory processing. Rewards act as a motivation for animals to be interested in achieving targets, which can be of natural origins, such as food, water, sex, or artificial as a substance (substance addiction study), electrical stimulation, etc. However, the natural rewards can quickly lead to satiation (satiety, stop craving, etc.), reducing motivation in the study of behavior and learning that needs to be evaluated in a relatively long process. Moreover, in order to train animals to learn to achieve a certain behavior, the study needs to be conducted continuously for a prolonged time.

Research on the stimulus factors to ensure reward creation for animals has been published. One of the most significant methods is intracranial electric self-stimulation (ICSS), which is a method for generating electrical stimuli that reward the brain.

Moreover, brain stimulation reward (BSR) has outstanding features in the study of memory and motivation which uses rewards as food, sex or substances [3, 8, 10] not affected by emotions, anxiety or rewards "satiation",... [6, 6, 9]. BSR is a type of stimulation that directly targets the reward system of the brain. ICSS-trained animals can continually perform tests to receive rewards for hours or even all day until exhaustion. In extreme conditions, animals may even choose BSR over food or warmth [12]. Furthermore, increasing stimulation intensity (dosage) correlates with an enhanced ICSS response. On the other hand, other types of reward, such as food, water, sex, or substances, will decrease the response and create an anxiety state or "satiation" of rewards.

2. PROBLEM

2.1. Research object

Animals: 07 male mice weighing between 26 and 29 g were obtained from the Central Institute

of Hygiene and Epidemiology.

Electrode implantation: monopolar stimulating electrodes (100 μm in diameter, stainless steel) were implanted into the medial forebrain bundle on both sides of the posterior lateral hypothalamic area for intracranial self-stimulation [2]. Animals were anesthetized with pentobarbital (40 mg/kg), two stainless steel unipolar electrodes, $\phi 100 \mu\text{m}$ (Inter Medical co., Ltd., Japan) were implanted into the medial frontal bundle at posterior lateral region of the hypothalamus to serve as intracranial stimulation electrode (anteroposterior, -2.3 mm; mediolateral, ± 0.7 to 0.75 mm; and dorsoventral, -5.3 to 5.4 mm) [4].

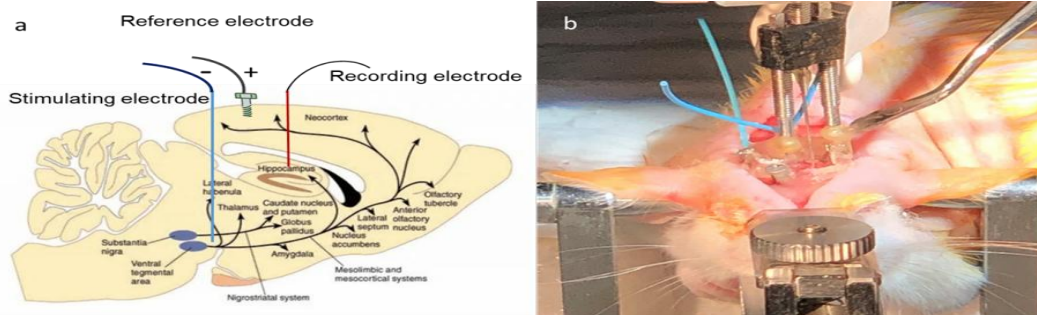


Figure 1. The illustration shows the implanted stimulating (a) [4] and recording electrodes (b).

This study uses a single metal microelectrode (monotrode) and 4 microelectrodes (tetraode) for collection. In addition, a recording micro-set of 8 single electrodes (for monotrode) or 2 collections of 4 microelectrodes (tetraode) ($\phi 17 \mu\text{m}$, California Fine Wire Company, USA) is designed to be implanted into CA1 region of hippocampus (coordinates in reference to Bregma point: anterior-posterior: -1.8 mm; medial-lateral: 1.8 mm; inferior: 1.4 mm) [4] (Figure 1).

2.2. Research tools

Description: The study utilized a rectangular box (size: length $\times$ width $\times$ height = 30 $\times$ 20 $\times$ 23 cm) featuring a small hole (diameter 1.5 cm) equipped with an optical sensor to count "nose-poking" instances (z reward area) described in Figure 2.



Figure 2. The recording chamber for the ICSS response and nose-poking behaviors of mice.

The mice were implanted with stimulating electrodes and placed in boxes to conduct research. The nose-poking task (NPT) was typically performed in 30-minute sessions. Between sessions, there are intervals to change the value of the stimulating parameter. The total test time is calculated as:

$$t_{LT} = t_s + t_{In} \quad (1)$$

Where t_{LT} is the total time, t_s is training time, also the total time of sessions, and t_{In} is resting for parameter adjustment.

When the mouse pokes its nose into the reward zone, the sensor sends a signal to the processor, which then activates the BSR excitation system.

- *Rewarding conditions*: Each time the mice performed the act of putting their nose in the reward area z it was considered a nose-poking.

- *Test stopping conditions*: When the mice complete the set test time, or the test time may vary depending on the purpose of the study.

2.3. Model and algorithm of NPT

The input consists of the nose-poking behavior, detected by an optical sensor. The sensor operates at a high logic status and switches to low logic upon a nose-poke. This signal is processed to count rewards and trigger stimulating pulses via a NI6501 interface that is described in the model in Figure 3. Each successful poke results in a rewarding pulse that creates interest for the mice to perform ICSS behavior.

2.3.1. Model of NPT

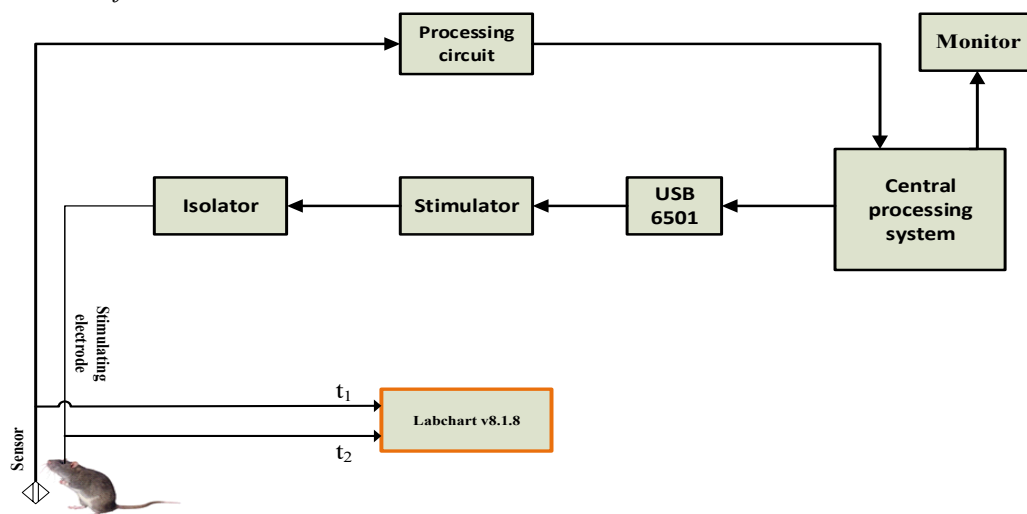


Figure 3. Model of system for stimulating and responding to nose-poke behavior and evaluating the stability and latency of the system for NPT by labchart Pro v8.1.8.

2.3.2. Model and algorithm of electrical stimulation of neurons with NPT

a) Significance of NPT

The flowchart of the excitation algorithm for the NPT is explained by the following steps:

The NPT algorithm is based on predefined reinforcement contingencies. Stimulation parameters, specifically intensity and frequency, are evaluated through empirical trials to validate previously simulated models [1].

Variables used in the algorithm include:

- Pt : Current number of rewards.
- $maxPt$: Maximum rewards per episode (condition to stop the program).
- $chammui$: Signal indicating the mouse touched the sensor.
- t : timer variable.
- $maxT$: maximum time for one exercise (condition to stop the program).
- $ptDelta$: reward for the delta period.
- $tDelta$: variable counting in delta time.
- $delta$: the amount of time to count the reward.
- $countInterval$: number of stops to adjust the parameter.
- $interval$: Pause time for parameter adjustment.

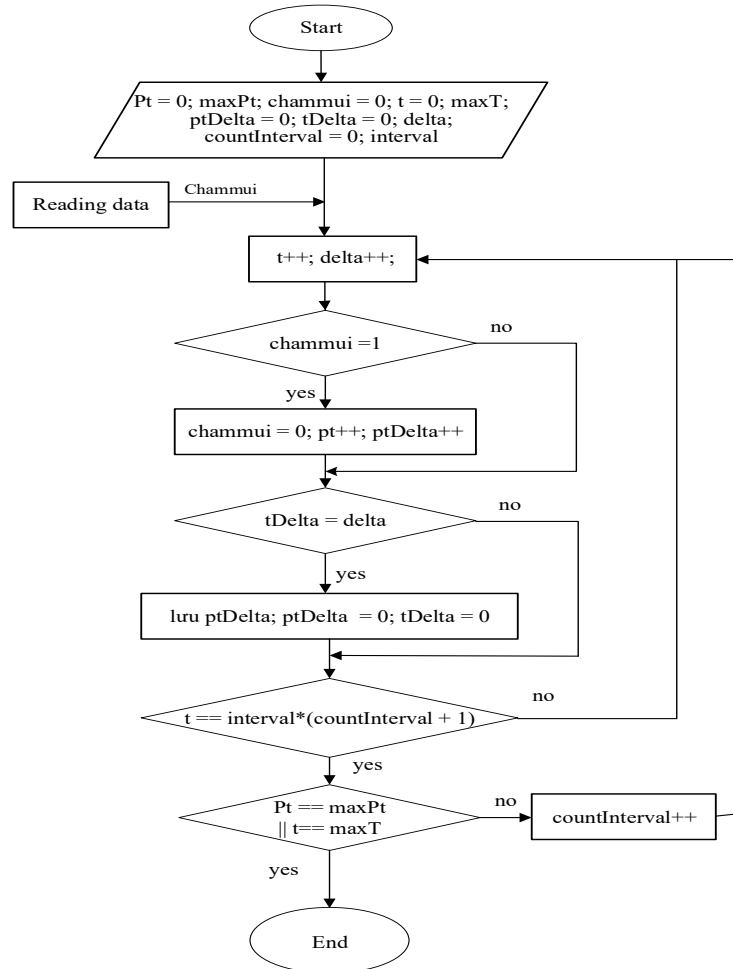


Figure 4. Flow chart of the NPT.

ICSS responses were recorded starting one week post-implantation. Each nose-poke triggered a 0.5 s pulse train consisting of 0.3 ms positive square cathodal pulses. Mice underwent training until behavioral stabilization was achieved (typically within 3 days) at 80 Hz and 80–90 μ A. Subsequently, the dependencies of response on stimulus intensity and frequency were investigated.

Over the following two days, the optimal stimulus intensity was determined by increasing the intensity in 10 μ A increments from 20 to 140 μ A at a fixed frequency [1]. The optimal intensity was defined as the first level eliciting a maximum response. During the final two days, ICSS responses to frequency variations were evaluated at an intensity equal to 70% of the previously determined optimum. The stimulation frequency was increased in steps of approximately 0.1 logarithmic units (16, 20, 25, 32, 40, 50, 63, 80, 100, 126, and 158 Hz). Each frequency step lasted 2 minutes, separated by a 30 second break. Data were monitored and recorded per minute by a central processing system for subsequent analysis.

b) Behavioral analysis

Mean responses were graphed using the Gompertz model [5]:

$$f(x) = a \times e^{-e^{(b-cx)}} \quad (2)$$

in which:

- $f(x)$: Nose-poking response frequency per unit of time.
- x : Independent variable, (e.g., stimulation frequency or intensity)

- a , b , c : Model parameters calculated to fit the empirical data which represents the asymptote, reflecting the maximum possible response rate.

To optimize the Gompertz model, the values of a , b , c and the bending point coordinates can be estimated with a being asymptotic on ($\lim_{x \rightarrow +\infty} f(x) = a$), which could be calculated:

$$X = \theta_{cr} = \frac{-\left\{\ln\left[-\ln\frac{cr}{a}\right] - b\right\}}{c} \quad (3)$$

Similar to the above formula, it is easy to calculate the M70, or M80 values, which are often used in some ICSS-related experiments [11].

- Stimulation intensity and frequency

The study was conducted on seven individual mice to evaluate the dependence of nose-poking response on stimulus pulse intensity, the mean value of nose-poking response performed in 1 minute and the standard deviation are shown in Figure 5A ($n = 6$, Table 1).

This study investigates the frequency-dependent nature of (ICSS) behavior (Figure 5B), specifically focusing on nose-poking responses in mice ($n = 7$, Table 2).

The actual recorded curve is consistent with the Gompertz model predictions and the response pulse trend of neuron electronic circuit modeling with stimulated pulse intensity and frequency values investigated in the simulation [1].

The actual response graph of the animal according to the stimulus intensity variation (fixed stimulus frequency at 70 Hz) and the response graph based on the Gompertz model show the similarity between the actual response and the theoretical model when the intensity level has not exceeded the optimal intensity (which is 100 μA in the graph of Figure 5A). For intensities exceeding the optimal level, there is a tendency for divergence between the actual response and the Gompertz model and the greater the stimulus intensity, the greater the divergence.

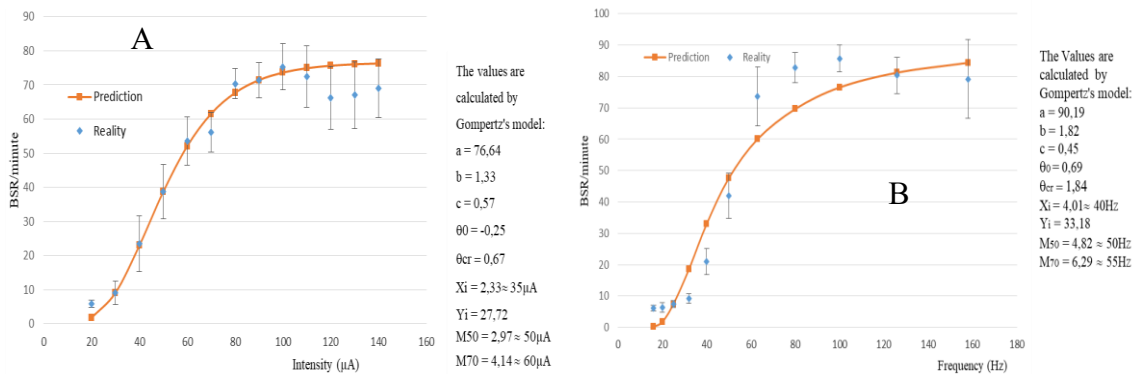


Figure 5. The dependence of nose-poking response on the stimulating intensity (A) and frequency (B) of stimulation.

At a fixed intensity (M70), the Gompertz model provides a robust framework for characterizing the transition from the stimulation threshold to the optimal frequency, effectively isolating reward-driven behavior from spontaneous baseline activity. Compared to simpler linear models or higher-order polynomial models (which might overfit and introduce unrealistic oscillations), the Gompertz model yields an optimally low (superior) AIC value. It effectively captures the non-linear biological reality without unnecessary mathematical complexity. Because the curve accurately traces the overall S-shape and matches the vast majority of data points tightly, the estimated R^2 for this fit is exceptionally high, likely falling between 0.95% and 0.98%.

It can be seen from Figure 5A that the average number of nose-poking behaviors in a minute depends on the intensities of the stimulating current (the blue lozenge dots - Table1) as compared to calculated values (the orange square dots) by the Gompertz model. The experiments were performed on seven mice (each mouse was repeated twice over two days). The variation shown in the graph is SE (standard error) for the statistical analysis of experiments.

3. RESULTS AND DISCUSSION

3.1. Results of nose-poking behaviors on the mice

The system continuously updated reward counts in "bins" (10 s) and "sessions" (2 minutes). Data analysis confirmed that mice response are highly dependent on stimulus intensity and frequency. Optimal parameters were found to be 100 μA and 100 Hz, as depicted in Figure 6.

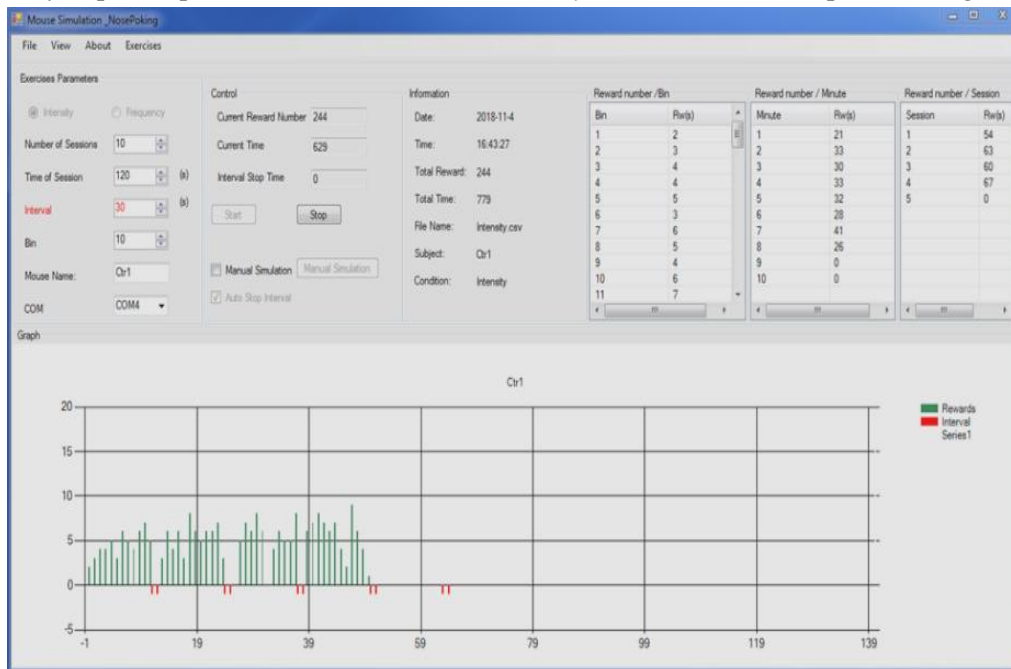


Figure 6. The program for simulating and recording of nose-poking response.

The data is saved and analyzed for the purpose of evaluating the animal's response to the value of the stimulus intensity and frequency. Update session-specific rewards by utilizing the blue columns. The range of values of the stimulus for the mice pokes its nose the most, or the maximum number of rewards, is the optimal range of stimulus values.

Table 1. The results of the nose-poking response depend on intensity (Times/ session).

Intensity (μA)	20	30	40	50	60	70	80	90	100	110	120	130	140
Subject	Day 1												
Ctr07	14	5	1	49	95	95	127	120	159	160	136	151	150
Ctr14	14	4	84	94	144	120	107	158	125	113	77	113	34
Ctr16	12	11	9	87	135	107	93	181	200	202	204	154	194
Ctr21	4	10	7	48	66	66	107	135	115	127	162	174	39
Ctr22	3	1	11	4	7	21	167	70	165	197	177	194	225
Ctr25	6	23	52	72	100	70	119	161	61	58	19	24	75

	Day 2												
Ctrl07	20	7	11	5	22	95	129	89	143	128	118	128	173
Ctrl14	9	42	11 4	13 8	111	121	116	111	146	121	123	127	166
Ctrl16	5	11	2	10 5	143	162	201	177	196	204	173	178	164
Ctrl21	16	17	76	65	120	165	158	189	160	186	147	135	166
Ctrl22	4	2	2	87	110	118	195	185	215	186	176	190	164
Ctrl25	15	8	83	41	148	109	134	87	124	60	68	34	74
$\bar{X}$	10	12	38	66	100	104	138	139	151	145	132	134	135
$\pm$ SE	2	3	11	11	13	11	10	12	12	14	15	15	17

Table 2. The results of the nose-poking response depend on frequency (times/ session).

Frequency (Hz)	16	20	25	32	40	50	63	80	100	126	158
Subject	Day 1										
Ctrl027	12	31	18	29	58	48	123	144	145	110	146
Ctrl029	15	5	5	25	10	29	233	165	184	191	253
Ctrl030	19	12	24	27	82	92	158	211	191	169	192
Ctrl032	19	6	5	12	16	74	168	161	145	155	143
Ctrl033	4	7	18	17	27	62	93	128	112	151	74
Ctrl034	12	32	20	19	23	117	147	204	195	214	179
Ctrl006	24	62	103	101	191	250	190	222	240	250	181
	Day 2										
Ctrl027	16	17	10	34	52	53	99	132	144	133	95
Ctrl029	5	3	10	3	32	59	219	228	259	258	271
Ctrl030	3	9	24	20	62	77	159	152	189	154	130
Ctrl032	13	15	30	11	41	115	202	190	183	176	179
Ctrl033	7	8	6	13	73	115	108	146	138	135	81
Ctrl034	24	6	8	13	29	167	178	187	188	202	157
Ctrl006	22	31	106	111	215	262	245	212	226	235	185
$\bar{X}$	13	13	28	29	72	121	173	178	185	185	127
$\pm$ SE	2	4	9	8	16	19	13	9	11	12	15

3.2. The evaluation of stimulation system

Use LabChart Pro v8.1.8 software to record the t_1 and t_2 times to evaluate the system latency for NPT exercises shown in Figure 3. The system delay for the NPT exercise Δt_{NPT} was the time interval between the time the mouse poked the nose and the time the mouse received the stimulus signal, averaging 60 ms. Whereas on an average, in 1 minute, the maximum number of rewards a mouse received was 82 rewards, the number of rewards that a mouse could receive was equal to 1.35 rewards in a 1 second period (under conditions of optimum electrical stimulus parameters

shown in Figure 5). To achieve 1 reward requires a minimum of $0.74 \text{ s} = 740 \text{ ms} \gg 60 \text{ ms}$. Thus, this is much smaller when compared to the time of reward acquisition.

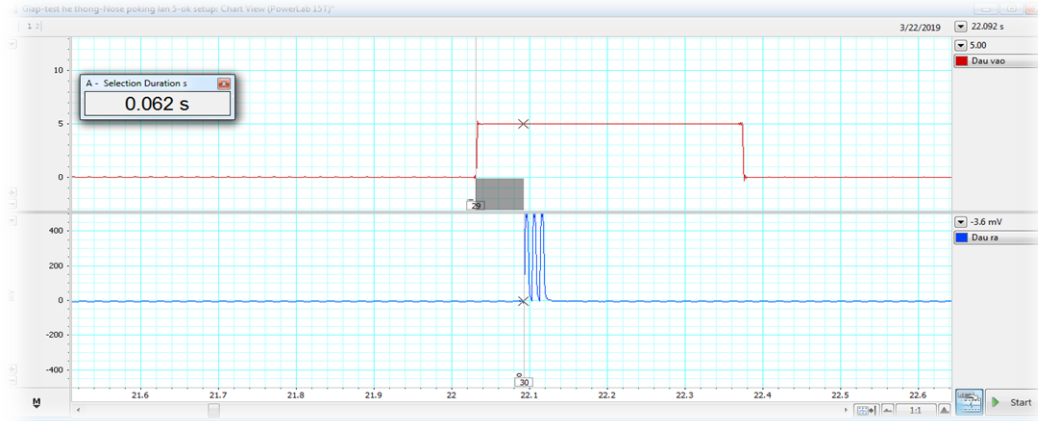


Figure 7. The illustration of pulses for the reward condition (red), reward delivery (blue), and the delay time of the system.

Table 3. The results of system latency time in NPT test (unit: s).

Turn 1	Turn 2	Turn 3	Turn 4	Turn 5	Turn 6	Turn 7	Turn 8	Turn 9	Turn 10
0.03	0.10	0.07	0.05	0.09	0.04	0.09	0.10	0.04	0.02
0.07	0.05	0.05	0.04	0.02	0.11	0.09	0.03	0.04	0.01
0.02	0.09	0.07	0.07	0.02	0.01	0.09	0.10	0.08	0.05
0.05	0.09	0.02	0.05	0.08	0.01	0.02	0.07	0.03	0.15
0.06	0.06	0.09	0.09	0.07	0.04	0.08	0.05	0.06	0.06
0.08	0.07	0.05	0.10	0.08	0.09	0.04	0.09	0.05	0.02
0.08	0.03	0.04	0.10	0.01	0.04	0.00	0.04	0.11	0.08
0.10	0.02	0.04	0.04	0.09	0.04	0.01	0.10	0.07	0.09
0.10	0.04	0.10	0.10	0.04	0.07	0.07	0.06	0.05	0.09
0.01	0.02	0.11	0.10	0.01	0.05	0.08	0.10	0.06	0.04
$\bar{X}_{\text{NPT}} = 0,06$		$SD = 0,03$		$\Delta t_{\text{NPT.Max}} = 0,15$			$\Delta t_{\text{NPT.Min}} = 0,01$		

4. CONCLUSIONS

The NPT exercise successfully determined optimal intensity ($100 \mu\text{A}$) and frequency (100 Hz) parameters for studying mouse learning ability.

The system demonstrated high stability, accurate algorithms, and minimal latency. These findings align with theoretical Gompertz models and previous research, establishing a solid foundation for further hippocampal place cell studies.

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TÓM TẮT

Đánh giá đáp ứng hành vi nhúng mũi của chuột thông qua hệ thống kích thích tế bào thần kinh

Trong nghiên cứu, đánh giá đáp ứng hành vi của động vật đối với kích thích, việc luyện tập thành thục một hành vi nhất định đóng vai trò quan trọng trong việc đánh giá đáp ứng của tế bào thần kinh đối với tín hiệu kích thích. Đối với những hoạt động hành vi của động vật, có nhiều phương pháp tiếp cận để nghiên cứu, đánh giá. Do đó, quá trình nghiên cứu cần nắm rõ các yếu tố tham số kích thích ảnh hưởng đến đáp ứng hành vi và xây dựng các thuật toán làm cơ sở xây dựng các bài tập cần hiểu rõ đặc điểm của đối tượng nghiên cứu cũng như xây dựng các điều kiện biên của bài toán. Nghiên cứu tìm hiểu hoạt động của hệ thần kinh trung ương ở mức tế bào, phân tử đang là một trong những xu hướng mới, đầy tiềm năng nhưng hiện nay ở trong nước chưa có cơ sở đào tạo, nghiên cứu nào xây dựng hệ thống kích thích và ghi hoạt động điện của tế bào thần kinh một cách hoàn chỉnh để đánh giá các hoạt động sinh lý của hệ thần kinh.

Từ khóa: Kích thích điện; Tế bào thần kinh; Tín hiệu kích thích; Đáp ứng hành vi.