Nicotine Addiction Models and Evaluation of Animal Behavior

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Abstract Nicotine is one of the most widely concerning components of cigarette smoke. Long-term intake of nicotine can lead to nicotine dependence that affects higher brain functions, which may involve anxiety, learning and memory impairments, and abnormal decision-making. To facilitate analyzing the molecular mechanisms of nicotine dependence, it is common to establish rodent nicotine dependence models to better understand the physiological changes. This paper summarizes and evaluates the application of animal behavior evaluation experiments in nicotine-dependent animal models, in order to provide a reliable reference for researchers to establish rodent nicotine-dependent models for evaluation.

Keywords Nicotine dependence; Animal addiction models; Animal behavior; Rodents; Evaluation method

Drug dependence involves social science, psychology, pharmacology, psychiatry, genetics, behavior and other disciplines[1]. Nicotine dependence (ND) is one of the most widespread types of drug dependence. According to *2020 China Reported Health Hazards of Smoking*, there are about 1 billion smokers in the world. In 2018, the smoking rate of people over 15 years old in China was 26.6%, with a total of more than 300 million people[2]. The composition of cigarette smoke is so complex that more than 7 000 substances have been found, and their neurobiological effects are mainly revealed by animal and cell tests with nicotine or smoke condensate[3]. Tobacco has a dual effect, and positive reinforcement occurs primarily at the beginning of dependence, including mild euphoria, enhanced memory, and emotional relaxation, while the negative effects of smoking are caused by withdrawal, including low mood, anxiety, and impaired memory, which combine to eventually lead to tobacco use and dependence[4].

The establishment of animal models with corresponding phenotypic characteristics is the basis of molecular mechanism research. Compared with other model organisms, rodents are featured by smaller size, docile temperament, easy feeding and short reproductive cycle, and the structure, function, metabolism and dependence characteristics are highly similar to human beings. Mice and rats are major rodents for nicotine-dependent models. By simulating the nicotine-dependent environment to build animal models, it is helpful to study the relationship and mechanism between the change of brain composition structure and behavior change, including activation of related brain regions, changes in the level of brain neurotransmitters, and changes in higher brain functions. In this paper, the application, advantages and disadvantages of various behavioral tests in the study of ND were analyzed in detail, so as to provide a useful reference for the study of addiction mechanism, brain function injury and behavior disorder of ND animal model.

Received: 2022-09-25 Accepted: 2022-11-21
Supported by Provincial and Ministerial Key Science and Technology Projects [110202101018(XX-04), 110202102014].
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1 Neurobiological Basis of ND Behavior

The formation of ND has the dual influence of psychological disorder and behavioral disorder. In the study of molecular mechanism of nicotine addiction, it is inconvenient to study human subjects. Therefore, animal models, with their advantages of simple feeding, fast reproduction rate, high homology with human beings and trainability, have become better alternative research objects, playing an important role in the study of ND. In previous studies, based on the establishment of multiple ND animal models, nicotine was found to induce the changes in ventral tegmental area (VTA), prefrontal cortex (PFC), hippocampus (HP) and other brain neurotransmitters related to learning and memory[5–6]. It mainly involves three major nervous systems: dopaminergic (DA) system, 5-serotonin (5-HT) system and glutamatergic (Glu) system, and the projec-

tion pathway is shown in Fig.1[7]. Nicotine causes changes in related neurotransmitters in the brain and activates intracellular signal transduction pathways by acting on nicotinic acetylcholine receptors (nAChRs), and contributes to changes in the morphological structure of neurons and advanced brain functions, ultimately leading to the formation of ND[8].

1.1 Reward effects of ND The reward effect induced by ND is mediated by the neurotransmitter DA in the dopaminergic system, and DA is mainly produced by substantia nigra (SN) and VTA regions in the midbrain basal ganglia. After the intake of nicotine, the body produces a sense of pleasure by causing a large amount of DA release in the NAc area, which is the main cause of DA[9]. The reward pathway induced by dopamine is called mesolimbic pathway or reward way [10]. In addition, the SN-striatum pathway projecting from SN to striatum is also associated with ND behavior [11]. Therefore, the abnormal release of DA in the dopaminergic system caused by nicotine is the main physiological incentive for the formation of ND [12]. Psychologically, it is mainly related to reinforcement effect, and will gradually increase the dose of nicotine to produce reward feelings, namely mental dependence behavior, which is the cause of relapse behavior [13-14]. Microdialysis experiments on ND rat models found that acute nicotine injection or chronic long-term nicotine exposure significantly increased the level of DA release in NAc, indicating that DA plays an important role in the process of ND[15]. The rewarding effect of nicotine is similar to agonists for DA receptors, which can activate D1 receptors to promote ND[16]. In rodents, the reward effect caused by nicotine is mainly manifested in the voluntary nicotine use preference, which is also a core feature of ND behavior[17-18].

1.2 Withdrawal effects of ND The withdrawal effect caused by nicotine withdrawal is mainly related to 5-hydroxytryptamine (5-HT) neurotransmitters. Anato-mically, serotoninergic neurons originate from brainstem raphe nuclei group and is usually associated with emotional diseases, suicide, impulsivity and drug abuse[19]. The neurotransmitter of this system is 5-HT, a monoamine inhibitory neurotransmitter, which plays an important role in maintaining synaptic plasticity, enhancing learning and memory, emotions, *etc*.[8]. 5-HT also modulates DA release by regulating 5-HT receptor on DA neurons[20], and 5-HT1A receptor on DA neurons of NAc promotes the release of DA [21]. The main contributor to the withdrawal effect is the inhibitory effect of 5-HT2A and 5-HT2C receptors on DA neurons in the striatum and midbrain on DA release, thus increasing anxiety symptoms[22-23]. Studies have shown that chronic nicotine exposure can cause increased 5-HT levels in the brain, which may be another risk factor for addiction and relapse[24], and 5-HT levels would decrease after withdrawal. Increasing 5-HT level is helpful to reduce relapse and other ND behaviors [8]. This also explains the cor-relation between anxiety and depression symptoms caused by quitting smoking and decreased activity of 5-HT neurons [25]. Emotional instability, hyperactivity and rest-lessness caused by ND are related to abnormal emotional behavior caused by 5-HT receptors[26], which mainly manifests in rodents as excessive combing, chewing, tremors, wet-dog shakes and tooth chatter. These behavioral characteristics can be used to evaluate the withdrawal behaviors in animal models[27].

1.3 Formation of ND behavior The formation of ND behavior also depends on the glutaminergic system, which is mainly involved in synaptic plasticity, cognition, learning and memory, and developmental processes of the brain[23]. Glutamic acid (Glu) is the most important excitatory neurotransmitter, 50% of which involve in synaptic transmission, while ionic Glu receptors are involved in many pathophysiological processes such as learning, memory, drug dependence and neurodegenerative diseases [28]. Rodent models are also useful for evaluating their ability to learn and remember after nicotine addiction. After nicotine enters the brain, it can act on PFC and cause Glu to release excitatory DA neurons, thus producing a reward effect[5]. On the other hand, nicotine can act on excitatory Glu neurons to form long-term potentiation (LTP). The brain is composed of neurons and glial cells connected to each other. Nicotine strengthens these connections through the glutaminergic system, causing structural changes in the brain. This is another neural basis for ND behavior.

1.4 Relationship between ND and other neurotransmitters Nicotine also interacts with other neurotransmitter systems in the brain. Studies showed that the release of norepinephrine (NE) in the rat midbrain increased after acute or chronic nicotine treatment in rodent models [29].

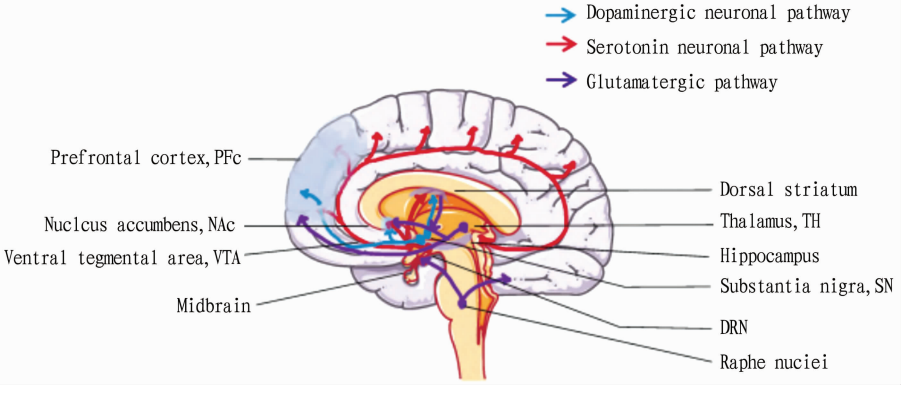

Fig.1 Three major nervous systems project to related areas of the brain

Nicotine regulates the secretion of NE in two ways: directly acting on the nAChRs of locus coeruleus; indirectly regulating NE secretion via GABA[5]. In addition, nicotine regulates synaptic plasticity throughout the brain through slow inhibition mediated by metabolic GABAB receptors (ABABRs)[30]. GABA receptor agonists can reduce the NE release induced by nicotine, and inhibit the formation of conditioned position preference (CPP) and nicotine self-medication behavior[31]. Endogenous cannabinoid receptors are also associated with nicotine addiction[32]. Endogenous cannabinoid receptor antagonists will weaken the activity of DA in the mesencephalic limbic system, thereby reducing the reward effect of nicotine [33]. In summary, a variety of neurotransmitters are involved in regulating the formation of ND behavior.

2 Behavioral Evaluation Experiment of ND Models

According to the neurobiological basis of nicotine addiction, the abnormal brain structure and function can be summarized into three characteristics: mental dependence, physical dependence and drug resistance. Mental dependence is mainly reflected in the initial stage, that is, animal emotional pleasure, increased autonomous activities, and memory enhancement to produce psychological craving. Physical dependence and drug resistance are mainly manifested as an enhanced response to mental activity stimulation in animals. Cessation of nicotine exposure leads to nicotine withdrawal symptoms, such as physical withdrawal signs, anxiety and depression-like behaviors, learning and memory deficits, and restlessness. Behavioral tests of ND animal models include the five aspects of behavioral preference, learning and memory, cognitive and decision-making function, emotion and exercise coordination ability. These experiments are helpful to establish and evaluate ND models (Fig.2).

2.1 Behavioral preference training experiments In behavioral preference training experiments, addictive drugs or controls are given to animals through specific instruments and training methods that simulate the addiction process, so that animals can establish a relationship between addictive substances and the environment and eventually produce preferences. Rodents are used to establish human-consistent ND behavior, to further study the dependence degree and molecular mechanism of this animal model. Behavioral preference training is mainly composed of self-administration model, self-sufficient light model, conditioned position preference model, behavioral sensitization model, *etc*. [34]. Rodent dependence on nicotine can be obtained by repeated injection of nicotine, long-term infusion of nicotine through osmotic micropumps, oral administration of nicotine, cigarette smoke exposure, nicotine atomization exposure, and exposure to e-cigarette aerosol containing nicotine. The evaluation criteria of behavioral preference training experiment are mainly evaluated from the four aspects of reliability, test validity, practicability and sensitivity.

2.1.1 Self-administration test. Self-administration test (SA) is the self-administration training of experimental animals through an operant conditioning test box, and its most notable feature is that it acquires nicotine in a completely autonomous manner (press rod or nose touch)[35]. Intravenous nicotine concentration in rats and mice ranges from 0.03 to 0.1 mL/kg; too low a concentration is not enough to form drug dependence, and too high a concentration will lead to nicotine aversion [36].

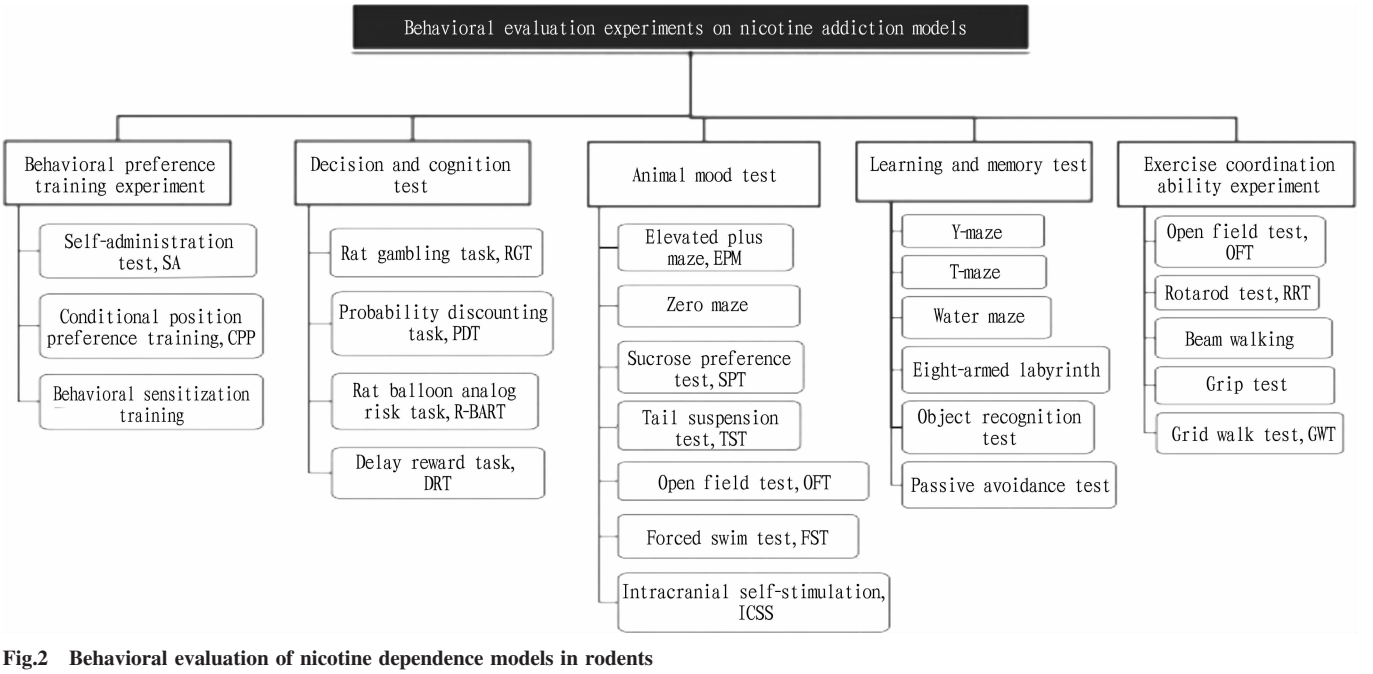

Fig.2 Behavioral evaluation of nicotine dependence models in rodents

Fixed ratio and progressive ratio are set in program training, to compare the variation in the number of pressure rods or nose contacts of animals at each stage in different procedures[37]. The withdrawal stage after addiction, namely break point, can evaluate the level of addiction and the drug seeking degree of animals[38]. Experimental animals in the intravenous self-administration drug model can well simulate the active drug seeking process and drug use behavior of drug users, and can accurately grasp the intake dose of drugs, which is a classic model for studying drug addiction. However, the disadvantage is that animal operation is difficult, and often has a high failure rate [39-40]. At present, nicotine self-administration models that can better simulate smoke inhalation have been developed, such as novel aerosol inhalation type nicotine self-administration model[41], which releases electronic cigarette aerosol containing different concentrations of nicotine (20, 40, 80 mg/mL) through animal autonomous nasal contact[42]. It can be used to evaluate different types of nicotine products containing nicotine or nicotine salts. The aerosol self-administration model can also be used to evaluate the ND enhancement properties of other complex chemical components and additives in cigarette smoke and electronic cigarette aerosol. However, the precise control of aerosol release stability and the accurate evaluation of actual drug dose ingested by animals are still technical difficulties.

Self-administration training can accurately count the frequency of administration. In terms of structural validity, it is similar to the human addiction mechanism, and fully reflects the subjective requirements of animals and has high reliability. However, it also has limitations, and can not well reflect the strong craving state when there is no drug available.

2.1.2 Conditional position preference. Conditional position preference (CPP) is an animal model based on classical conditioning, which is used to establish a relationship between the injected substance and the environment by giving animals a reward or aversion stimulus or control solvent. It usually consists of 3 compartments. The environment of the two compartments must be different, contrasting in space configuration, color, floor and even other senses, with the middle compartment used to adapt to the environment. During nicotine training, nicotine is injected in the white box and normal saline is injected into the black box. After multiple operations, animals can form a conditional match between the environment and nicotine[43]. At present, the nicotine CPP models successfully established can induce CPP in rats within the dose range of 0.1–1.4 mg/kg[44]. In addition, it is also an important means to study addiction by using CPP training to give reward stimulation to study the neural pathways of reward system[44]. Rodents can construct ND models through CPP training, to evaluate its influence on learning and memory [45] and the preference differences caused by gender differences[46-47].

The construction principle of CPP animal model is based on the low-level reflex activity innervated by the lower nervous system. The training is easier and cheaper, and the experiment period is short. Therefore, CPP animal model has been widely applied in the construction of addictive behavior model, which is more suitable for the experiment with a large amount of data and relatively discrete data.

2.1.3 Behavioral sensitization training. Behavioral sensitization training is mainly based on the theory of "neurostimulation sensitization", that is, a transformation of psychological activity from "like" to "need" for addictive drugs[8]. The establishment process of behavioral sensitization includes three stages, namely, formation stage, transformation stage and expression stimulation stage. During the formation stage, the animals were injected with nicotine every day for 5–7 d. During the transformation stage, drug administration was stopped and withdrawal lasted for 4–7 d, during which animal behavior changed to sensitization. In the expression stimulation stage, behavioral sensitization was triggered by nicotine injection in animals, and the spontaneous activity increased significantly. Through 3 periods of training, the animals shifted from high activity to nicotine to behavioral sensitization. Animal behavior sensitization training will produce addictive exercise effects such as increased exercise behavior[48]. The increase of DA level in the brain caused by nicotine through direct or indirect means is directly manifested as increased animal activity[49]. Rodents usually show an increase in exercise behavior and repeated behavior (such as chewing, shaking head and tickling) in a relatively narrow space. In the experiment, motor coordination ability can be further used to evaluate the level of addiction.

At present, the theory of addiction sensitization, which has good theoretical and structural basis, has been widely recognized. Due to simple operation, short experimental period and intuitive evaluation method, it has been a good ND and anti-addiction efficacy evaluation.

2.2 Decision and cognition test Long-term nicotine exposure can lead to changes in brain structure that will affect the neural structure and brain function related to decision-making. Risk decision experiments can be used to detect the impaired decision-making ability of animals. The decision and cognition tests of rodents include rat gambling task (RGT), probability discounting task (PDT), delay reward task and rat balloon analog risk task (R-BART)[50]. The basis of good decision behavior includes reward, emotion, learning and memory, exercise execution and other nervous systems[8]. Brain function impairment may lead to cognitive disorders, behavioral abnormalities and other phenomena. "From cognitive improvement to relapse prevention" has been regarded as the idea of auxiliary treatment of addiction disorders[51]. Neurotransmitters such as DA and 5-HT

also participate in the regulation of risk decision making and various cognitive functions[52]. PDT test has proved that addiction can cause damage to orbitofrontal cortex and frontal cortex, thus affecting decision. Decision cognitive tests can evaluate the decision behavior and cognitive level of rats and mice in nicotine preference training[53], and impulsive selection in delay reward tasks can predict the re-use of nicotine after withdrawal[54].

2.3 Animal mood test Withdrawal syndromes composed of physical, emotional and cognitive symptoms caused by smoking cessation are risk factors for relapse[55]. These withdrawal symptoms are most evident within the first week after cessation of nicotine exposure, with anxiety and depression-like behaviors, as well as learning and memory deficits lasting for months[56]. Emotional symptoms in rodents are more subtle than somatic symptoms, so assessment requires a series of behavioral analyses to reveal the emotional changes associated with withdrawal. Mood tests mainly include elevated plus maze (EPM), zero maze or O-maze, sucrose preference test (SPT), tail suspension test (TST), forced swim test (FST), intracranial self-stimulation (ICSS), *etc*.[57], and the emotional state of animals with ND can reflect the severity of withdrawal symptoms. EPM is used to measure the stress of nicotine exposure in test animals[58], which is easy to test and record, and does not require pre-training, but relies on the exploratory nature of rodents[59]. The reduced time spent exploring the open arm of O-maze reflects anxious behavior after nicotine intake[60]. SPT and FST are used to test anhedonia and depression in animals during nicotine withdrawal[61-62], and sucrose preference decreases in mice during nicotine withdrawal[63]. FST test shows that the increase of despair behavior in the nicotine withdrawal model is manifested as no exercise. In order to rule out insufficient movement performance, endurance swimming test could be supplemented[62]. Anhedonia is one of the symptoms of withdrawal, and ICSS can be used to assess anhedonia during withdrawal[64], as well as nicotine exposure and nicotine administration during acute and chronic withdrawal[65].

2.4 Learning and memory test The nAChRs of α7 and α4β2 are highly expressed in the brain regions important for learning and memory, such as hippocampus and cerebral cortex[66]. Withdrawal symptoms such as learning and memory deficits would persist from day 1 to day 90 after exposure to e-cigarette aerosol or smoking cessation in mice[67]. Long-term exposure to nicotine can lead to desensitization of nAChRs, which has certain effects on the learning and memory ability of animals. Maze tests are commonly used experimental instruments to study learning, memory, spatial orientation and cognitive ability of rodents, including T-maze, Y-maze, water maze, eight-armed labyrinth, *etc*.[68]. Such a maze is frequently used to evaluate cognitive decline, hippocampal structural damage, and the effects of nicotine on spatial memory in ND animal models, and significant deficits in attention and working memory have been found in male mice exposed to nicotine[69]. Eight-armed labyrinth evaluation shows that mice exposed to nicotine show deficits in spatial and reference memory[70]. In addition, different learning and memory methods, such as improved elevated maze test, situational fear conditioning test, passive avoidance test and object recognition test, can be used to evaluate the learning and memory ability during ND and withdrawal.

2.5 Exercise coordination ability experiment Low doses of nicotine increase exercise activity and sensitize mice and rats. Sensory tests, exercise tests, reflection tests and beam balance tests are performed on rodents to assess the level of neurological deficits and nerve damage[71]. The evaluation tests include open field test (OFT), rotarod test (RRT), beam walking, grip test, grid walk test (GWT), *etc*. OFT is used to evaluate the occurrence of various behaviors of experimental animals in a wide environment [72]. Through record analysis, it is found that after nicotine withdrawal, animals have abnormal states such as trembling, shaking, jumping, retrea-ting, nodding, chewing, standing, scratching, teasing, rotating, digging, abdominal contraction and licking[56]. RRT and beam walking can measure exercise coordination and balance, also is used to test possible side effects of nicotine ingestion on exercise coordination and assess function defects of sensorimotor nerve [73]. Grip test evaluates the influence of nicotine on animal limb muscles, and grid walking test is mainly used to evaluate exercise abnormalities and behavioral defects[74]. Most of these tests require automated equipment and software to perform video analysis.

2.6 Experimental data acquisition and analysis methods Behavioral experiments need objective judgment of indicators, and the normal activities of animals should not be affected during the observation process, so as to ensure the correctness of experimental results. At present, data collection for animal behavior testing can be carried out by means of instruments and equipment, including data information, video information, sound information, *etc*. (Fig.3)[75]. Image processing is capable of more flexible, fine and comprehensive measurement of animal behaviors, and has become the most popular behavior recognition technology[76]. Peleh *et al*.[77] invented a tracking and analysis tool to observe the behavior of a group of mice. A variety of software analysis systems and algorithm designs have been developed, and the basic process is shown in Fig.3. Motion trajectories and various behavioral characteristics of rodents in the divided areas can be analyzed online, and heat maps and charts of motion trajectories can be generated to improve the accuracy and efficiency of animal behavior experiments.

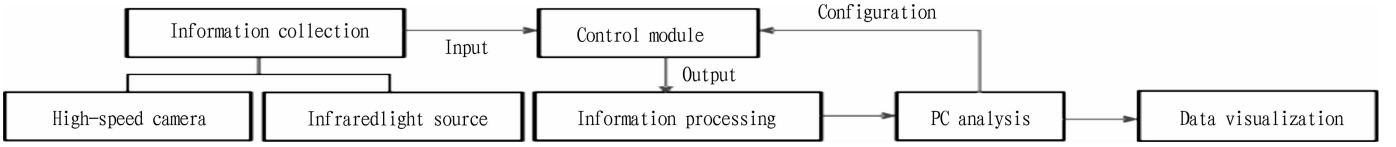

Fig.3 Experimental data acquisition and analysis process

3 Conclusions

The mechanism of nicotine addiction is very complex. How to evaluate the dependence degree of animal model and its withdrawal symptoms is a problem worthy of further discussion. To study the mechanism of ND formation, maintenance and relapse, the primary challenge is to develop higher and more effective animal models of dependence and withdrawal and behavioral evaluation models. The ND models and animal models of nicotine withdrawal are established by various exposure methods, which could evaluate from the degree of addiction, the severity of withdrawal symptoms and the effect of drug treatment. In behavioral tests, in addition to detection methods and technical parameters, there are many factors that can affect the experimental results, which should be greatly concerned. First, the choice of administration method should be closer to the actual use of tobacco products. Second, we should constantly develop more perfect test methods and automated recording and analysis instruments and equipment for behavioral science, so as to reduce external influences and improve the objectivity and accuracy of data collection and analysis. Besides, most existing studies have used only adult male rodents to assess the effects of nicotine withdrawal and drug therapy. In order to improve the predictive validity of various ND models, more effective animal dependence and withdrawal models can be developed to study ND and its symptoms after withdrawal. In addition, exploring the differences between ND and gender and age will have greater significance for the treatment of human ND, which may promote the development of new drug therapies and reduce the symptoms of ND and withdrawal. It is expected to fully reveal the molecular mechanisms of relevant brain regions, neural circuits and neurotransmitters.

References

[1] HYMAN SE. Addiction: a disease of learning and memory [J]. Am J Psychiatry, 2005, 162(8): 1414–1422.

[2] WANG C, XIAO D, CHI H. Summary of China smoking hazards health report 2020 [M]. Beijing: People's Medical Publishing House, 2021.

[3] GENG ZL, ZHANG J, GE YH, *et al*. Correlation between endogenous harmful components in mainstream cigarette smoke and chemical constituents in tobacco leaves[J]. Chin J Applied Ecol, 2015, 26(5): 1447–1453.

[4] MANNING V, VERDEJO-GARCIA A, LUBMAN DI. Neurocognitive impairment in addiction and opportunities for intervention[J]. Curr Opin Behav Sci, 2017(13): 40–45.

[5] HU YF. A magnetic resonance imaging study of brain structural changes in nicotine dependence[D]. Wuhan: Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences, 2021.

[6] SCHULTZ W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey[J]. J Neurophysiol, 1986, 56(5): 1439–1461.

[7] IKEMOTO S, QIN M, LIU ZH. Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area [J]. J Neurosci, 2006, 26 (3): 723–730.

[8] HAN L. The neural mechanism of nicotine-induced abnormality indecision-making[D]. Hefei: University of Science and Technology of China, 2018.

[9] COOPER S, ROBISON AJ, MAZEI-ROBISON MS. Reward circuitry in addiction[J]. Neurotherapeutics, 2017, 14(3): 687–697.

[10] LÜSCHER C. The emergence of a circuit model for addiction[J]. Annu Rev Neurosci, 2016, 39: 257–276.

[11] NUTT DJ, LINGFORD-HUGHES A, ERRITZOE D, *et al*. The dopamine theory of addiction: 40 years of highs and lows [J]. Nat Rev Neurosci, 2015, 16(5): 305–312.

[12] KLEIN MO, BATTAGELLO DS, CARDOSO AR, *et al*. Dopamine:functions, signaling, and association with neurological diseases[J]. Cell Mol Neurobiol, 2019, 39(1): 31–59.

[13] SUBRAMANIYAN M, DANI JA. Dopaminergic and cholinergic learning mechanisms in nicotine addiction[J]. Ann N Y Acad Sci, 2015, 1349(1): 46–63.

[14] GIPSON CD, REISSNER KJ, KUPCHIK YM, *et al*. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity [J]. Proc Natl Acad Sci USA, 2013, 110 (22): 9124–9129.

[15] RAHMAN S, ZHANG J, CORRIGALL WA. Effects of acute and chronic nicotine on somatodendritic dopamine release of the rat ventral tegmental area: *in vivo* microdialysis study [J]. Neurosci Lett, 2003, 348(2): 61–64.

[16] SPINA L, FENU S, LONGONI R, *et al*. Nicotine conditioned singletrial place preference: selective role of nucleus accumbens shell dopamine D_1 receptors in acquisition [J]. Psychopharmacology, 2006, 184(3): 447–455.

[17] SOLINAS M, BELUJON P, FERNAGUT PO, *et al*. Dopamine and addiction: what have we learned from 40 years of research [J]. J Neural Transm, 2019, 126(4): 481–516.

[18] BAIK JH. Dopamine signaling in reward-related behaviors [J]. Front Neural Circuits, 2013(7): 152.

[19] COOLS R, ROBERTS AC, ROBBINS TW. Serotoninergic regulation of emotional and behavioural control processes [J]. Trends Cogn Sci, 2008, 12(1): 31–40.

[20] YAN QS. Activation of 5-HT2A/2C receptors within the nucleus accumbens increases local dopaminergic transmission[J]. Brain Res Bull, 2000, 51(1): 75–81.

[21] VAN DEN HEUVEL LEIGH L. Stahl's essential psychopharmacology: Neuroscientific basis and practical applications (4^{th} edition)[J]. J Child Adolesc Ment Health, 2014, 26(2): 157–158.

[22] KASWAN NK, MOHAMMED IZHAM N, TENGKU MOHAMAD T, *et al*. Cardamonin modulates neuropathic pain through the possible involvement of serotonergic 5-HT1A rec eptor pathway in CCI induced neuropathic pain mice model [J]. Molecules, 2021, 26(12):

3677.

[23] POPESCU A, MARIAN M, DRAˇ GOI AM, *et al.* Understanding the genetics and neurobiological pathways behind addiction (Review)[J]. Exp Ther Med, 2021, 21(5): 544.

[24] BALASUBRAMANI PP, CHAKRAVARTHY VS, RAVINDRAN B, *et al.* A network model of basal Ganglia for understanding the roles of dopamine and serotonin in reward-punishment-risk based decision making [J]. Front Comput Neurosci, 2015, 9: 76.

[25] MÜLLER CP, HOMBERG JR. The role of serotonin in drug use andaddiction [J]. Behav Brain Res, 2015(277): 146–192.

[26] MARIA ALEXANDRA STANESCU A, TOTAN A, MIRCESCU D, *et al.* Assessment of suicidal behavior in dermatology (Review) [J]. Exp Ther Med, 2020, 20(1): 73–77.

[27] BALFOUR DJK, MUNAFÒ MR. The neuropharmacology of nicotine dependence [M]. Switzerland: Springer International Publishing, 2015.

[28] GRILLNER P, SVENSSON TH. Nicotine-induced excitation of midbrain dopamine neurons *in vitro* involves ionotropic glutamate receptor activation [J]. Synapse, 2000, 38(1): 1–9.

[29] YUEN EY, WEI J, LIU W, *et al.* Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex[J]. Neuron, 2012, 73 (5): 962–977.

[30] LI SP, PARK MS, KIM JH, *et al.* Chronic nicotine and smoke treatment modulate dopaminergic activities in ventral tegmental area and nucleus accumbens and the γ-aminobutyric acid type B receptor expression of the rat prefrontal cortex[J]. J Neurosci Res, 2004, 78(6): 868–879.

[31] LI X, SLESINGER PA. GABAB receptors and drug addiction: psychostimulants and other drugs of abuse [M]. Behavioral Neurobiology of GABAB Receptor Function. Cham: Springer International Publishing, 2020.

[32] LIU Q, YU J, LI X, *et al.* Cannabinoid receptor GPR55 activation blocks nicotine use disorder by regulation of AMPAR phosphorylation [J]. Psychopharmacology, 2021, 238(11): 3335–3346.

[33] FANG Q, LI FQ, LI YQ, *et al.* Cannabinoid CB1 receptor antagonist rimonabant disrupts nicotine reward associated memoryin rats [J]. Pharmacol Biochem Behav, 2011, 99(4): 738–742.

[34] WEI Z, ZHANG X. Similarities and differences in diagnostic criterion [J]. Adv Exp Med Biol, 2017(1010): 105–132.

[35] FUNADA M. Animal models for evaluating of drug dependence liability [J]. Nihon Rinsho, 2010, 68(8): 1459–1464.

[36] GELLNER CA, BELLUZZI JD, LESLIE FM. Self-administration of nicotine and cigarette smoke extract in adolescent and adult rats[J]. Neuropharmacology, 2016, 109: 247–253.

[37] ZHAO C, ZHANG L, MA BM, *et al.* Application of progressive ratioin self-administration model [J]. Chin J Drug Depend, 2011, 20 (3): 169–172.

[38] DING ZM, GAO Y, SENTIR AM, *et al.* Self-administration of cotinine in wistar rats: comparisons to nicotine [J]. J Pharmacol Exp Ther, 2021, 376(3): 338–347.

[39] FLORES RJ, URIBE KP, SWALVE N, *et al.* Sex differences in nicotine intravenous self-administration: a meta-analytic review [J]. Physiol Behav, 2019, 203: 42–50.

[40] CLEMENS KJ, STUART A, FERGUSON SG. Pre-quit nicotine decreases nicotine self-administration and attenuates cue and drug induced reinstatement [J]. J Psychopharmacol, 2019, 33(3): 364–371.

[41] LALLAI V, CHEN YC, ROYBAL MM, *et al.* Nicotine e-cigarette vapor inhalation and self-administration in a rodent model: sex and nicotine delivery-specific effects on metabolism and behavior [J]. Addict Biol, 2021, 26(6): e13024.

[42] MONTANARI C, KELLEY LK, KERR TM, *et al.* Nicotine e-cigarette vapor inhalation effects on nicotine & cotinine plasma levels and somatic withdrawal signs in adult male Wistar rats [J]. Psychopharmacology, 2020, 237(3): 613–625.

[43] WILAR G, SHINODA Y, SASAOKA T, *et al.* Crucial role of dopamine D2 receptor signaling in nicotine induced conditioned place preference [J]. Mol Neurobiol, 2019, 56(12): 7911–7928.

[44] LIN R, LIANG J, WANG R, *et al.* The raphe dopamine system controls the expression of incentive memory [J]. Neuron, 2020, 106(3): 498–514.

[45] DALAVERI F, NAKHAEE N, ESMAEILPOUR K, *et al.* Effects of maternal separation on nicotine induced conditioned place preference and subsequent learning and memory in adolescent female rats [J]. Neurosci Lett, 2017(639): 151–156.

[46] LIU J, TAO X, LIU F, *et al.* Behavior and hippocampal epac signaling to nicotine CPP in mice [J]. Transl Neurosci, 2019(10): 254–259.

[47] YARARBAS G, KESER A, KANIT L, *et al.* Nicotine-induced conditioned place preference in rats: Sex differences and the role of mGluR5 receptors[J]. Neuropharmacology, 2010, 58(2): 374–382.

[48] LI PY, JING MY, WU N, *et al.* Behavioral animal models of addiction [J]. Chin J Drug Abuse Prev Treat, 2021, 27(2): 118–124.

[49] NATSHEH JY, ESPINOZA D, BHIMANI S, *et al.* The effects of the dopamine D2/3 agonist quinpirole on incentive value and palatability-based choice in a rodent model of attention-deficit/hyperactivity disorder [J]. Psychopharmacology, 2021, 238 (11): 3143–3153.

[50] ORSINI CA, HERNANDEZ CM, BIZON JL, *et al.* Deconstructing value-based decision making via temporally selective manipulation of neural activity: insights from rodent models[J]. Cogn Affect Behav Neurosci, 2019, 19 (3): 459–476.

[51] REZAPOUR T, DEVITO EE, SOFUOGLU M, *et al.* Perspectives on neurocognitive rehabilitation as an adjunct treatment for addictive disorders: from cognitive improvement to relapse prevention[J]. Prog Brain Res, 2016(224): 345–369.

[52] JENNI NL, LARKIN JD, FLORESCO SB. Prefrontal dopamine D1 and D2 receptors regulate dissociable aspects of decision making via distinct ventral striatal and amygdalar circuits [J]. J Neurosci, 2017, 37(26): 6200–6213.

[53] ORSINI CA, BLAES SL, DRAGONE RJ, *et al.* Distinct relationships between risky decision making and cocaine self-administration under short and long-access conditions [J]. Prog Neuropsychopharmacol Biol Psychiatry, 2020 (98): 109791.

[54] DIERGAARDE L, PATTIJ T, POORTVLIET I, *et al.* Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats [J]. Biol Psychiatry, 2008, 63(3): 301–308.

[55] ELHASSAN S, BAGDAS D, DAMAJ MI. Effects of nicotine metabolites on nicotine withdrawal behaviors in mice [J]. Nicotine Tob Res, 2017, 19(6): 763–766.

[56] CHELLIAN R, BEHNOOD-ROD A, BRUIJNZEEL DM, *et al.* Rodent models for nicotine withdrawal[J]. J Psychopharmacol, 2021, 35(10): 1169–1187.

[57] YANKELEVITCH-YAHAV R, FRANKO M,

HULY A, *et al*. The forced swim test as a model of depressive-like behavior[J]. J Vis Exp, 2015(97): 52587.

[58] KRAEUTER AK, GUEST PC, SARNYAI Z. The elevated plus maze test for measuring anxiety-like behavior in rodents [J]. Methods Mol Biol, 2019, 1916: 69–74.

[59] BIAŁA G, KRUK M. Influence of bupropion and calcium channel antagonists on the nicotine-induced memory-related response of mice in the elevated plus maze [J]. Pharmacol Rep, 2009, 61(2): 236–244.

[60] NGUYEN C, MONDOLONI S, LE BORGNE T, *et al*. Nicotine inhibits the VTA-to-amygdala dopamine pathway to promote anxiety [J]. Neuron, 2021, 109(16): 2604–2615.

[61] ZHAO D, XU X, PAN L, *et al*. Pharmacologic activation of cholinergic alpha7 nicotinic receptors mitigates depressive-like behavior in a mouse model of chronic stress [J]. J Neuroinflammation, 2017, 14(1): 234.

[62] RIBEIRO-CARVALHO A, LIMA CS, NUNES-FREITAS AL, *et al*. Exposure to nicotine and ethanol in adolescent mice: effects on depressive-like behavior during exposure and withdrawal[J]. Behav Brain Res, 2011, 221(1): 282–289.

[63] ALKHLAIF Y, BAGDAS D, JACKSON A, *et al*. Assessment of nicotine withdrawal-induced changes in sucrose preference in mice [J]. Pharmacol Biochem Behav, 2017(161): 47–52.

[64] HARRIS AC, TALLY L, MUELKEN P, *et al*. Effects of nicotine and minor tobacco alkaloids on intracranial-self-stimulation in rats [J]. Drug Alcohol Depend, 2015(153): 330–334.

[65] BAUZO RM, BRUIJNZEEL AW. Animal models of nicotine withdrawal: intracranial self-stimulation and somatic signs of withdrawal [J]. Methods Mol Biol, 2012(829): 257–268.

[66] XU Y. The alterations of nicotinic acetylcholine receptors in type 2 diabetic brain and the molecular mechanism of enhancing the function of α7 nicotinic acetylcholine receptors to ameliorate spatial learning and memory impairment in diabetic mice [D]. Guiyang: Guizhou Medical University; 2020.

[67] PONZONI L, BRAIDA D, CARBONI L, *et al*. Persistent cognitive and affective alterations at late withdrawal stages after long-term intermittent exposure to tobacco smoke or electronic cigarette vapour: behavioural changes and their neurochemical correlates [J]. Pharmacol Res, 2020, 158: 104941.

[68] MILADI GORJI H, RASHIDY-POUR A, FATHOLLAHI Y. Effects of morphine dependence on the performance of rats in reference and working versions of the water maze [J]. Physiol Behav, 2008, 93(3): 622–627.

[69] ZHANG L, SPENCER TJ, BIEDERMAN J, *et al*. Attention and working memory deficits in a perinatal nicotine exposure mouse model [J]. PLoS One, 2018, 13(5): e0198064.

[70] GAWEL K, GIBULAE, MARSZALEK-GRABSKA M, *et al*. Assessment of spatial learning and memory in the Barnes maze task in rodents-methodological consideration [J]. Naunyn Schmiedebergs Arch Pharmacol, 2019, 392 (1): 1–18.

[71] SONG Y, YANG Y, CUI Y, *et al*. Lipoxin A4 methyl ester reduces early brain injury by inhibition of the nuclear factor kappa B(NF-κB) -dependent matrix metallopeptidase 9 (MMP-9) pathway in a rat model of intracerebral hemorrhage [J]. Med Sci Monit, 2019(25): 1838–1847.

[72] KRAEUTER AK, GUEST PC, SARNYAI Z. The open field test for measuring locomotor activity and anxiety-like behavior [J]. Methods Mol Biol, 2019(1916): 99–103.

[73] SHI X, BAI H, WANG J, *et al*. Behavioral assessment of sensory, motor, emotion, and cognition in rodent models of intracerebral hemorrhage [J]. Front Neurol, 2021(12): 667511.

[74] ZHU W, GAO Y, WAN J, *et al*. Changes in motor function, cognition, and emotion related behavior after right hemispheric intracerebral hemorrhage in various brain regions of mouse [J]. Brain Behav Immun, 2018(69): 568–581.

[75] SHI LJ, WANG SB, ZHAO J, *et al*. Experimental system of animal behavioristics based on LVRT system [J]. Chin J Sci Instrum, 2021, 42(1): 137–145.

[76] PACUTA A, AGAR A, KOCíKOV B, *et al*. Time matters. Locomotor behavior of *Lacerta viridis* and *Lacerta agilis* in an open field maze[J]. Acta Ethol, 2018, 21(2): 91–99.

[77] PELEH T, BAI X, KAS MJH, *et al*. RFID-supported video tracking for automated analysis of social behaviour in groups of mice [J]. J Neurosci Methods, 2019(325): 108323.