ՀՏԴ 159.97

Հոգեբանություն

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PhD.

STRESS-FREE COLLECTION OF CONTINUOUS RODENT BEHAVIORS AND ULTRASOUNDS VOCALIZATION (USV) DATA FOR AUTISM STUDY AND THE NEED FOR MULTI-DIMENSIONAL AND MULTI-FUNCTIONAL MEASUREMENT IN PRECLINICAL STUDY FOR AUTISM RESEARCH

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ԱՈՒՏԻԶՄԻ ՈՒՍՈՒՄՆԱՍԻՐՄԱՆ ԱՆԸՆԴՀԱՏ ՌԵԺԻՄՈՒՄ ԿՐՇՈՂՆԵՐԻ ՎԱՐՔԻ ՏՎՅԱԼՆԵՐԻ ՀԱՎԱՔԱԳՐՈՒՄԸ ԵՎ ԱՌԱՆՑ ՍԹՐԵՍԻ ԳԵՐԶԱՅՆԱՅԻՆ ՎՈԿԱԼԻԶԱՑՄԱՆ ՎԵՐԼՈՒԾՈՒԹՅՈՒՆԸ, ԱՈՒՏԻԶՄԻ ՈՒՍՈՒՄՆԱՍԻՐՄԱՆ ՆԱԽԱԿԼԻՆԻԿԱԿԱՆ ՀԵՏԱԶՈՏՈՒԹՅՈՒՆՆԵՐՈՒՄ ԲԱԶՄԱԶԱՓ ԵՎ ԲԱԶՄԱՖՈՒՆԿՑԻՈՆԱԼ ՉԱՓՈՒՄՆԵՐԻ ԱՆՀՐԱԺԵՇՏՈՒԹՅՈՒՆԸ

Աուտիզմը որին բնորոշ են աուտիկ սպեկտրի խանգարումները՝ԱՍԽ, հոգեկան հիվանդություն է, որն առաջ է գալիս ժառանգական ֆոնի և միջավայրի բարդ փոխազդեցության հետևանքով: Ախտանիշաններն են սոցիալական հաղորդակցման, ուշադրության և տեղեկատվության մշակման անբավարարությունը, կենտրոնացման դեֆիցիտը, շփման խանգարումները, գերակտիվությունը, ցավի նկատմամբ ոնկպատնակության նվազումը, վարքի կրկնվող տարբեր ձևերը: Ներկայումս աուտիզմը կամ ԱՍԽ-ն, ախտորոշում են վարքային ֆենոտիպացման, այլ ոչ թե նեյրոկենսաբանական թերությունների միջոցով: Կենդանիների վրա կատարված փորձերը նպատակ են հանգարումների ծագումնաբանության հասկացմանը՝ արդյունավետ ախտորոշման ստանդարտի և բուժման եղանակների որոնման նպատակով: Վարքային ցուցիչների ֆենոտիպերի փորձարկված «ոսկե ստանդարտներն» անհրաժեշտ են վարքային ցուցիչների քանակական գնահատման համար: Այնուամենայնիվ, ամբողջ աշխարհում, նկատվում է աուտիզմով հիվանդների թվի զգալի էքսպոնենցիալ աճ՝ 1980-ական թվականների համեմատությամբ: ԱՍԽ-ի ուսումնասիրման համար վճռական նշանակություն ունեն կենդանիների վարքային ֆենոտիպերը և վարքի հետագոտման

հնարավորություններ: Մի քանի տասնամյակների ընթացքում ԱՄՆ հետազոտման նպատակով օգտագործվում են կենդանիների երեք մոդելներ՝ խոցման մոդելներ, գենետիկական և շրջակա միջավայրի ազդեցության մոդելներ: Կենդանու վարքի վրա այս մոդելներից յուրաքանչյուրի ազդեցության չափը որոշվում է վարքի հավաքակազմի (խմբի) վերլուծության կամ վարքային ֆենոտիպի վերլուծությամբ: Հաշվի առնելով աուտիստիկ հիվանդության բարդություններ և նրա էթիմիոլոգիան՝ հետազոտողները հաճախ կենտրոնանում են նրա տարբեր առանձնահատկությունների վրա՝ կենդանական մոդելների օգտագործմամբ: «Metris B.V.» (Մետրիս, Նիդեռլանդներ) ընկերությունը հետազոտողներին առաջարկում է ԱՄՆ ուսումնասիրման համար օգտագործել բազմաչափ և բազմաֆունկցիոնալ չափանիշներ:

Բանալի բառեր՝ աուտիսիկ սպեկտրի խանգարումներ՝ ԱՄՆ, վարքային ֆենոտիպացում, բազմաչափ և բազմաֆունկցիոնալ չափանիշներ:

Л. Багдасарян (Metris BV)

СБОР ДАННЫХ ПОВЕДЕНИИ ГРЫЗУНОВ НЕПРЕРЫВНОМ РЕЖИМЕ И АНАЛИЗ УЛЬТРАЗВУКОВОЙ ВОКАЛИЗАЦИИ (USV) БЕЗ СТРЕССА ДЛЯ ИССЛЕДОВАНИЯ АУТИЗМА, НЕОБХОДИМОСТЬ МНОГОМЕРНЫХ И МНОГОФУНКЦИОНАЛЬНЫХ ИЗМЕРЕНИЙ В ДОКЛИНИЧЕСКИХ ИССЛЕДОВАНИЯХ ДЛЯ ИССЛЕДОВАНИЯ АУТИЗМА

Заболевание аутизмом имеет расстройство аутистического спектра (РАС) - это психическое заболевание, которое возникает из-за сложного взаимодействия между генетическим фоном и окружающей средой. Симптомами являются дефицит социального взаимодействия, нарушения коммуникации, обработки информации и внимания, дефицит концентрации, гиперактивность, уменьшения чувствительности к боли и несколько повторяющихся форм разных поведении. В настоящее время аутизм или РАС диагностируют на основе поведенческих фенотипированием, а не нейробиологических дефектов. Модели на животных использовались для понимания этиологии расстройства, чтобы найти хороший стандарт для постановки диагноза и для поиска способов лечения. Проверенные «золотые стандарты» поведенческих фенотипов для количественной оценки поведенческих параметров необходимы для поведенческих исследований. Тем не менее, в каждой стране наблюдается значительный экспоненциальный рост числа случаев аутизма во всем мире по сравнению с 1980-ми годами. Для исследователей РАС решающее значение имеют поведенческие фенотипы животных и возможности исследования поведения. В течение нескольких десятилетий в исследованиях РАС используются только 3 модели животных - модели поражения, генетические модели и модель влияния окружающей среды. Влияние на поведение животных измеряется в каждой из этих моделей с использованием набора (группы) поведенческого анализа или

анализа поведенческого фенотипа. Учитывая сложность аутистического заболевания и его этиологию, исследователи часто сосредотачиваются только на отдельных особенностях аутизма при использовании животных модели. Metris B.V. (Нидерланды) предлагает исследователям использовать многомерные и многофункциональные параметры для поведенческого исследования РАС.
Ключевые слова: расстройство аутистического спектра (РАС), поведенческое фенотипирование, многомерные и многофункциональные параметры.

Autism disease has Autism Spectrum Disorder (ASD) is a psychiatric disease that is caused due to the complex interplay between genetic and environmental background. The symptoms are social interaction deficits, communication impairments, information processing and attention, concentration deficits, hyperactivity, less sensitive to pain and several repetitive behaviors. Autism disease or ASD is currently diagnosed based on the behavioral phenotypic traits, rather than neurobiological defects. Animal models have been used to gain insight into the etiology of the disorder, in order to find a good standard to drive the diagnosis, and to search fortreatments. Validated "gold standard" behavioral phenotyping testes for quantifying behavioral parameters is essential for behavioral research.However, there is a significant exponential increase in the number of autism cases around the world compared to the 1980s in every country. For the ASD researchers become crucial animal behavioral phenotyping and behavioral research possibilities. Over several decades only 3 animal models are used in ASD studies - lesion models, genetic models and environmental influences model. The effects on the behavior of the animals are measured in each of these models using a set (group) of behavioral analysis or behavioral phenotyping.Given the complexity of autism disease and its etiology, researchers often focus only on single features of autism when using animal models. Metris B.V. (The Netherlands) proposes researchers to use multi-dimensional and multi-functional parameters for ASD behavioral study.
Key words: Autism Spectrum Disorder (ASD), behavioral phenotyping, multi-dimensional and multi-functional parameters

Abstract

Autism disease has Autism Spectrum Disorder (ASD) is a psychiatric disease that is caused due to the complex interplay between genetic and environmental background. The symptoms are social interaction deficits, communication impairments, information processing and attention, concentration deficits, hyperactivity, less sensitive to pain and several repetitive behaviors.

Autism disease or ASD is currently diagnosed based on the behavioral phenotypic traits, rather than neurobiological defects. Animal models have been used to gain insight into the etiology of the disorder, in order to find a good standard to drive the diagnosis, and to search for treatments.

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Over several decades only 3 animal models are used in ASD studies - lesion models, genetic models and environmental influences model. The effects on the behavior of the animals are measured in each of these models using a set (group) of behavioral analysis or behavioral phenotyping.

Given the complexity of autism disease and its etiology, researchers often focus only on single features of autism when using animal models. Metris B.V. (The Netherlands) proposes researchers to use multi-dimensional and multi-functional parameters for ASD behavioral study.

In this thesis, various types of animal models for ASD are critically reviewed for their use to mimic the complexity of the developmental biology of the disorder. Face validity, construct validity and predictive validity of these models are evaluated, as along with the rodent behavioral analysis and phenotyping. This is done using Metris B.V. products (namely: LABORAS System for behavioral analysis, Sonotrack system for USV recording and analysis, Sonotrack Automatic Ultrasounds Call Classification software and SmartChamber cabin for isolation and for the control of environmental conditions). Future directions point towards a multimodal approach, in which the animal body as one functioning system is emphasized. However, these experiments should be designed in a way that the animal is minimally restricted in performing its natural behavior, therefore Stress-free collection of continuous rodent behaviors and constant environmental conditions are crucial.

Current trends in the Pharmaceutical industry requires new translational approaches for pre-clinical test. Those aspects can be achieved by animal experiments in which not only one variable (e.g. one behavior) at the time is analyzed but rather a multidimensional approach (physiological parameters + several behaviors + Ultra Sound Vocalization (USV)+ different parameters from study) is applied. Therefore, automation and integration of different measuring technologies become the crucial aspects in this process. Combining knowledge, we narrow down the funnel towards not only a gold standard model, but also a gold standard behavioral phenotyping strategy, thereby increasing the translational capacity of the animal model.

Epidemiology of Autism

The epidemiology of autism is the study of the incidence and distribution of Autism Spectrum Disorders (ASD). A 2020 review of global prevalence estimates of autism spectrum disorders found a median of 68 cases per 10,000 people1.

However, there is a significant exponential increase in the number of autism cases around the world compared to the 1980s in every country. ASD averages a 4.3:1 male-to-female ratio in diagnosis. The number of children known to have autism has increased dramatically since the 1980s, at least partly due to changes in diagnostic practice; it is unclear whether prevalence has actually increased and as-yet-unidentified environmental

[1] Data & Statistics on Autism Spectrum Disorder | CDC

risk factors cannot be ruled out (mobile telephone networks, Wi-Fi antennas, processed food, drinking toxic symptoms, binge drinking toxic effects, medicine toxic effects, vaccination.). Little research exists to support associations with specific environmental exposures. Although the cause of autism is not known, it is suggested to be genetic, environmental influences and it appears that exposure to three teratogens can be a risk factor in developing this disorder. These three are thalidomide, valproic acid and ethanol.

There is no known single cause for autism, but it is generally accepted that it is caused by abnormalities in brain structure or function. Brain scans show differences in the shape and structure of the brain in autistic versus non-autistic children. Researchers are investigating a number of theories, including the link between heredity, genetics and medical problems. In many families, there appears to be a pattern of autism or related disabilities, further supporting a genetic basis to the disorder. While no one gene has been identified as causing autism, researchers are searching for irregular segments of genetic code that autistic children may have inherited. It also appears that some children are born with a susceptibility to autism, but researchers have not yet identified a single "trigger" that causes autism to develop. Other researchers are investigating the possibility that under certain conditions, a cluster of unstable genes may interfere with brain development resulting in autism. Still other researchers are investigating problems during pregnancy or delivery as well as environmental factors such as viral infections, metabolic imbalances, mobile telephone, Wi-Fi antennas and exposure to environmental chemicals.

Autism is severe developmental disorder that develop in the first three years of life. It is characterized by impaired social interaction, deficits in verbal and nonverbal communication, information processing and attention, concentration deficits, hyperactivity, less sensitive to pain and unusual, repetitive or severely limited activities, behaviors & interests. It affects males more than females.

In children with autism, repetitive behaviors and gastrointestinal problems may be connected, new research has found1. The study found that increased severity of other autism symptoms was also associated with more severe constipation, stomach pain and other gut difficulties. In children with autism, repetitive behaviors and gastrointestinal problems may be also connected

Children with autism spectrum disorder are more likely than their typically developing peers to experience a range of gastrointestinal abnormalities, including chronic diarrhea, constipation, food sensitivities and abdominal pain. These symptoms have been associated with higher levels of irritability and aggressive behavior, but less is known about their relationship with other autism spectrum disorder symptoms. Gastrointestinal problems are a major concern for many children with autism and we still have a lot to learn about the complicated gut/brain axis.

Patients affected by autism may also present several medical diseases. A correct medical treatment of these diseases may improve the quality of life, well-being, and behavior of the patients affected and may also positively impact upon the behavioral and educational treatments. An accurate recognition of the medical diseases associated with autism may also help to identify subgroups of ASDs with homogeneous phenotypic and genetic characteristics and may thus foster a better understanding of the possible pathogenetic

[1] *Autism study suggests connection between repetitive behaviors, gut problems: Severity of GI symptoms, other autism symptoms also associated – Author Misti Crane - Science Daily*

mechanisms responsible for ASD's. Gastrointestinal (GI) disorders are among the medical pathologies associated with autism.

At present, there is no scientific research and conclusive data to unrelate environmental exposure (Wi-Fi, Mobile telecommunication, 5G networks, electromagnetic radiation) & ASDs and GI disorders & ASDs.

It is possible that different mechanisms and different genes are implicated in a group of subjects with ASDs and GI & immune system disorders. Variables considered in the gut-brain axis model include bowel inflammatory diseases, food allergies, increased intestinal permeability, the interaction between the immunological system and the blood-brain barrier, microglia, astrocytes, and neuronal modulation; however, more studies are necessary to clarify these relationships.

Animal models of Autism

The development of an animal model of autism is one approach researchers use to study potential causes of autism. Animal models provide the opportunity to decipher the relationships between the nervous system and behavior and they are an obligatory step for drug test. There are several animal models for ASD study (Lesions models, Genetic models, Environmental factor models).

Given the complexity of autism disease and its etiology, researchers often focus only on single features of autism when using animal models.

I propose researchers to use multi-dimensional and multi-functional parameters for ASD preclinical study.

Criteria for an effective animal model:

Effective animal models should meet at least three types of validity, which are:

1) Face validity, which means that the model has strong analogies to the endophenotypes (i.e., quantifiable components in the genes-to-behaviors pathways of the human syndrome)

2) Construct validity, which states that the model has the same biological dysfunction that causes the human disease, such as a gene mutation or anatomical abnormality

3) Predictive validity, meaning that the model has an analogous response to treatments that prevent or reverse symptoms in the human disorder.

Only when these criteria are met, a relationship can be deduced between the origin of the pathology and the behavioral phenotype resulting from it. For each validity step, there are several animal models. However, another important criterion is that the phenotyping assays that are applied to assess the behavioral effects of the manipulations, take into account the animal's natural/normal behavior. There is also a commercial factor in the selection of animal models for research.

Neuropathology of GABA Receptors

Rodents, most especially mice, are excellent animal models of autism because they have similar social relationships and neuroscience. When exposed to prenatal valproate (VPA) during pregnancy, the mice are born with basic deformities and the developmental delays seen symptomatically in humans. This is all comparable and easier to study since the lifespan of mice and most rodents is shorter, so being able to understand the genetics, minute effects, and test methods to reduce the onset of the disorder allows for researchers to develop new treatment methods quickly and effectively to help humans. Additionally, these rodents may trace back particular models to how the developmental delays occur in relation to GABA. GABA is a neurotransmitter that is generally seen as inhibitory, but

prior to birth and in early development of the brain it is often excitatory while neurons establish proper brain chemistry. During development there are specific times, called critical periods, where the brain is more capable of acquiring neural connections which usually leads to new behavioral and psychological skills. GABA's change from excitatory to inhibitory, as well as other neurotransmitter changes during these critical developmental stages can impact the development the brain goes through. If the critical period is early, growth can be limited, slowed, or even stunted early on. Additionally, if it is later, the brain's development is measured as complete incorrectly which may limit its ability to improve connectivity. Overall, the brain's circuitry and communication are often limited or poor within ASD, so using rodent models to study these limitations and where they come about increases researchers understanding of the disorder and potential ways to prevent it.

Environmental models and factors of ASD

Looking at the environmental factors of autistic spectrum disorder in rodents helps us to understand the neuropathology of the disorder which can be compared to humans. Environmental factors have been studied in animal rodent models and have been seen to influence brain development and play a role in CNS neuropathology. Since environmental factors can occur at any time during the developmental process, there is much variability in the neural and behavioral phenotype of autism. The environment can cause unknown changes in brain development of rodents because they don't all live in the same habitat and therefore might develop different changes to their brain than what is expected.

When constructing models that mimic the development of Autism or syndromic ASD, the behavioral symptoms are the point of departure in validating these models, rather than the resemblance of the underlying mechanism. This means that face validity is often provided, but construct validity is hardly ever provided using these models. The most widely used models to demonstrate the influence of environmental factors on the etiology of ASD are; 1) prenatal exposure to neurotoxins, like valproic acid (VPA), thalidomide or sevoflurane; and 2) prenatal or perinatal exposure to viral agents, like Borna virus, Rubella virus, and influenza. These models are often used to further explore the neurochemistry of ASD. For example, the inflammations in the brain caused by prenatal exposure to viral agents are believed to cause immune dysfunction, which may be similar to the mechanism that causes ASD. Finally, a growing body of evidence suggest that alterations in neuropeptide mechanisms, such as the opiate or glutamatergic system, are associated with ASD etiology.

Table 1. Frequently used models and behavioral tests illustrating environmental influences on ASD etiology

Animal		Autism exposure on behavior		Behavioural tests		
Rodent model	**model type**	**ASD-like behaviours**	**LABORAS sysem**	**SONOTRACK system**	**SmartChamber**	**Sonotrack Automated Call Clasification for mice (SACC)**
VPA	Neurotoxin	• Reduced exploratory behaviour	• Laboras normal behaviors			
	exposure	• Lower sensitivity to painful stimuli	• Laboras formaline test	USV Recording and Analysis		
		• Decreased social interactions	• Laboras sociability cage, 3 Chaber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
		• Increased repetitive behaviours	• Laboras Grooming; Scratching behaviours			
		• Increased anxiety	• Laboras Dark/Light cage; Laboras Freezing	USV Recording and Analysis	low USV	
Borna disease	Viral agent	• Decreased social interactions	• Laboras sociability cage, 3 Chaber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
virus	exposure					
uvenile	Housing	• Reduced social exploration	• Laboras sociability cage, 3 Chaber test	USV Recording and Analysis		
isolation	strategy	• Reduced anogenital sniffing	• Laboras normal behaviors			
		• Reduced approach/following		USV Recording and Analysis		SACC for mice and Sonotracl Play-back system
Opiate	Neuropeptide	• Reduced social contact	• Laboras sociability cage, 3 Chaber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
	administration	• Lower sensitive to painful stimuli	• Laboras formaline test			
		• Extreme persistence of behaviour	• Laboras normal behaviors			
Glutamate	Neuropeptide	• Slightly improved sociability	• Laboras sociability cage, 3 Chaber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
antagonist	administration	• Reduced self-grooming	• Laboras Grooming; Scratching behaviours			
BALB mice						
BTBR mice						
Oxytocin	Neuropeptide	• Increased non-sexual social interactions	• Laboras sociability cage, 3 Chaber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
	administration	• Increased autogrooming	• Laboras Grooming; Scratching behaviours			

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Genetic models and phenotypic factors of ASD

Although the neuropathology of ASD is not yet clear, twin and family genetic studies provide evidence that the disorder has a genetic component. There have been six autism-related genes that are linked to the X chromosome when it comes to autistic spectrum disorder. The first gene that has been linked to autism is the Fragile X mental retardation gene (Fmr1). For example, rodents with this gene exhibit elevated cortical spine densities that are similar to those found in autism as well as decreased social behaviors. Another gene that has been linked to autism is methyl-CpG- binding protein type 2 gene (MECP2). In the rodent models that have MECP2 disruption, the rodents are usually normal up until the sixteenth week of age and then they start to develop extreme anxiety in the field, reduced nest building, and poor social interactions which are all symptoms of autism. The third and fourth genes that have been linked to autism are neuroligin (NLGN) 3 and 4 genes. Researchers found that mutations in the NLGN 3 and 4 genes lead to loss of neuroligin processing to stimulate the formation of synapses which is a feature of autistic spectrum disorders. The fifth and sixth genes that are linked to autism are the tuberous sclerosis genes (TSC1 and TSC2). Mutations in one of these two genes cause multiple benign tumors to grow in multiple tissues like the brain. Lastly, many of the abnormalities found in autistic spectrum disorders involve the mTOR signaling pathway, the GABA - containing neurons, and the immune system.

The development of genetic models for ASD started with the emergence of single gene knock-out mouse models. An example is the oxytocin (OT)-KO and OT receptor (OTR)-KO mice. The candidate genes deleted in these models were identified through human studies, from which oxytocin appears to be an important factor in autism etiology. The OT-KO and OTR-KO mouse models are used to characterize the behavioral impact of genetic defects in oxytocin regulation, following the ‘gene to behavior’ approach. Thus, in these types of models the mechanism of the disorder is leading, i.e. investigating whether these genes cause the behavioral defects, rather than the behavioral symptoms of the disorder. These mice displayed deviant ultrasonic vocalizations as infants, as reduced calling rates

were observed in OT-KO pups compared to wild type pups when separated from their mother.

Table 2. Frequently used genetic mouse models and behavioral phenotyping assays in ASD research

Animal	Autism exposure on behavior		Behavioural tests		
Mouse model	**ASD-like behaviours (Adopted from Silverman 2010)**	**LABORAS sysem**	**Sonotrack system**	**SmarChamber**	**Sonotrack Automated Call Clasification for mice (SACC)**
BTBR	• Reduced reciprocal social interactions	• Laboras sociability test (sociability cage)/3 chamber test	USV Recording and Analysis		SACC for mice and Sonotracl Play-back system
	• Reduced sociability	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis; Sonotracl Play-back	low USV	
	• Increased repetitive self-grooming	• Laboras spontaneous grooming behaviour			
	• Reduced social transmission of food preference (STFP)	• Laboras food preference test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
	• Ultrasonic vocalizations elevated in pups and reduced in adults		USV Recording and Analysis; Sonotracl Play-back	low USV	SACC for mice and Sonotracl Play-back system
	• Unusual ultrasonic vocalization call categories in pups and adults				
C58/J	• Reduced sociability	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis; Sonotracl Play-back	low USV	
	• High level of repetitive motor stereotypies	• Laboras Schratching, Circling , Grooming, Climbing , Locomotion behavior			
	• Increased repetitive self-grooming	• Laboras Grooming behavior			
BALB	• Reduced sociability	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis; Sonotracl Play-back	low USV	
	• No genotype differences in preference for social novelty		USV Recording and Analysis	low USV	
	• Reduced reciprocal social interactions	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis; Sonotracl Play-back	low USV	SACC for mice and Sonotracl Play-back system
	• Reduced ultrasonic vocalizations in adolescent same-sex social interaction		USV Recording and Analysis; Sonotracl Play-back	low USV	SACC for mice and Sonotracl Play-back system
	• Reduced place-conditioned social reward	•Laboras Dark/Light cage, Laboras position distribution			
	• Reduced social learning during social distress	LABORAS future development			
Fmr1 KO	• Increased social approach	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis		
	• Reduced reciprocal social interactions	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
	• No genotype differences in sociability	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system
	• No genotype differences in preference for social novelty	• Laboras food preference test; Laboras Dark/Light cage	USV Recording and Analysis		
	• Low sociability dependent on genetic background	• Laboras sociability test (sociability cage)/ 3 chamber test	USV Recording and Analysis	low USV	SACC for mice and Sonotracl Play-back system

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Lesion models of ASD

Understanding human neurodevelopmental disorders often requires adequate models to understand the overall nature of the disorder and the general impacts the disorder makes on the brain itself. Naturally each disorder has different implications when it comes to genetic makeup, phenotypically and genotypically, and generally this impacts particular brain regions. In Autism Spectrum Disorder (ASD) it is generally seen in reduced developmental growth within the brain, and more specifically reduced gray matter within the medial temporal lobe (MTL), which is where the amygdala and hippocampus are located. This is critical in understanding Autism because this region of the brain controls emotions and learning, which is symptomatically linked to ASD. In addition, this supports the need for animal models that establish a greater understanding of what effects these particular brain regions and genetics have on development, and if there are measures, we can take to prevent the onset of the disorder.

The behavior disappearing or appearing after the brain lesion may be the result of other brain structures, which are related to the lesioned area. Especially when lesioning regions of the limbic system, which is thought to play a crucial role in autism pathology, one should be careful with interpreting the behavioral results. The limbic system serves as an inhibitor on many other brain functions; therefore, lesions in this region may induce disinhibition of other behaviors, resulting in all kinds of behaviors that are no longer controlled. These behaviors are then incorrectly associated with the lesioned area. Furthermore, since artificial lesions are likely to be caused differently than lesions caused by accidents or diseases, lesioning is less helpful in providing insight into the first etiological steps of a given disorder.

An important aspect to consider when discussing the validity of lesion models is the plasticity of the brain; when one area is damaged, another area may take over its functions. This holds especially for lateralized brain areas. This complicates the interpretation of lesion studies even further, since the exact function of a lesioned area cannot be ascertained once that function has been taken over by another area, and therefore no abnormal behavior is observed in the animal. This may be the case especially in the early lesion models described in this chapter. Since the brain is still developing at the time the lesion is conducted, it may be possible that parts of the functions are taken over by other brain regions. However, lesions may have to be conducted early in neurodevelopment, since ASD is hypothesized to arise at a very early stage.

Neuropathology of the Underdeveloped Synapse

Autism Spectrum Disorder (ASD) is caused by developmental delays that cause the brain to have lower connectivity within particularly important regions. The synapses within the brain have critical importance in development in young children, especially during their critical period. Autistic brains often have delayed or early critical periods, causing complications within the brain's developmental stages and ability to create stronger synapses for basic communication and stimulus recognition. Furthermore, the brain's lessened development and cognitive delays are usually observable within the genetics and grey matter within the brain.

Rodent models have been established as good examples because their brains are akin to humans in makeup. Additionally, they have similar social interactions and relationships that humans have, which shows the social development symptoms often used to diagnose ASD. Rodents when used as models are compared to their normal developed brains, but to replicate ASD, the rodents are lesioned prior to birth using prenatal valproate (VPA). The rodents then experience similar symptoms and developmental changes that occur with human's with ASD. Human's with ASD are identified to have a single-gene mutation at Neuroligin-3, or NL-3 R451C. These particularly simple changes to the rodents and human brains impact them greatly in their ability to develop properly.

Rodent behavioral models for ASD study

In ASD study, mouse models are very important. There are two main ways of measuring Autism Spectrum Disorders in mice: Social Communication and Repetitive behaviors.

Behaviors measured in rodent models include approach to olfactory pheromones emitted by other rodents, approach to familiar and new conspecifics, reciprocal social interactions, ultrasonic vocalizations, communal nesting, sexual and parenting behaviors, territorial scent marking, repetitive behavior such as compulsive grooming and scratching, hyperactivity, locomotion behavior, circling, pain, anxiety, cognition and aggressive behaviors. Social interaction is measured by how the rodent interacts with a stranger rodent introduced in the opposite side of a test box.

The following behavior models for ASD can be studded by using Laboras, Sonotrack and SmartChamber system:

Reciprocal social interactions: (Laboras Sociability cage for mice/ 3 chamber test, Laboras Position distribution, Sonotrack recording and analysis system for USV analysis, Sonotrack Play-Back system)

Ultrasonic vocalizations: (Sonotrack recording and analysis system for mice/rats, Sonotrack Play-Back system, Sonotrack Automated Call Classification software for mice, SmartChamber for mice/rats)

Communal nesting: (Sonotrack recording and analysis system for mice/rats, Sonotrack Play-Back system, Sonotrack Automated Call Classification software for mice, SmartChamber)

Sexual and parenting behaviors: (Sonotrack recording and analysis system for mice/rats, Sonotrack Automated Call Classification software for mice, Sonotrack Play-Back system, SmartChamber for mice/rats)

Territorial scent marking: (Laboras system position distribution)

Repetitive behaviors: (Laboras system Grooming for mice/rats, Scratching for mice/rats, Circling for mice/rats, Rearing for mice/rats, Climbing for mice, Sonotrack recording and analysis system for mice/rats)

Hyperactivity: (Laboras system Locomotion behavior, Circling, Rearing, Grooming, Scratching, Climbing, Sonotrack recording and analysis system for USV study)

Stereotyped behaviors: (Laboras system Locomotion behavior, Circling, Rearing, Grooming, Scratching, WDS, HS/HT, Sonotrack recording and analysis system for USV study)

Pain: (Laboras system normal behaviors and formalin pain test/ Hindlimb licking behavior, Sonotrack recording and analysis system for USV study, Sonotrack Automated Call Classification software for mice, SmartChamber for mice/rats)

Anxiety: (Laboras system normal behaviors and Dark/light cage test, Position distribution, Sonotrack recording and analysis system for USV study, Sonotrack Automated Call Classification software for mice, SmartChamber for mice/rats)

Cognition: (Laboras system, Sonotrack recording and analysis system for mice/rats, Sonotrack Play-Back system)

Aggressive behaviors: (SmartChamber for mice/rats, Sonotrack recording and analysis system for mice/rats, Sonotrack Automated Call Classification software for mice)

Behavior performance test in mice and rats: (Laboras system 2 food choices or 2 drinking choices - preference test)

Pop isolation cage (Box): (Laboras system, position distribution, Sonotrack recording and analysis system for USV study, Sonotrack Automated Call Classification software for mice,)

Fear conditioning protocol: (Laboras system, Startle response and Freezing behavior, Sonotrack recording and analysis system for mice/rats, Sonotrack Play back system, Automated Call Classification software for mice, SmartChamber for mice/rats)

The latent inhibition of conditioned response (LI): (Laboras system with extension cognitive test, Sonotrack recording and analysis system for mice/rats, Sonotrack play-back system, Sonotrack Automated Call Classification software for mice)

Sleep study: (Laboras system Immobility, Freezing behavior, with SleepSign software)

Future possibilities for developments (Laboras system - vertical jumping, back-flipping, digging, marble burying behaviors algorithm development, extension social preference and Sonotrack system - Automated Call Classification Software for Rats)

A high-throughput, stress-free and home-cage monitoring approach to do so would be using Laboras system for behavioral study and Sonotrack system for ultrasounds vocalization study.

LABORAS: Completely automatic recognition, recording and analysis of the behavior and tracking of small laboratory rodents (rats, mice), based on the analysis of force and energy.

LABORAS is an advanced system that automates behavior scoring of small laboratory animals. The system tracks positions and identifies more than 18 validated stereotypical and normal behaviors in mice and rats. Laboras does not use video or infra-red beams! The use of LABORAS system is applied in preclinical research for deep behavioral study. The recognition engine is so sensitive that it recognizes random/behavioral short signals from 0.1 seconds and high frequency behaviors 30Hz. The device recognizes, records and analyzes animal behavior completely automatically (Head Twitches/Head Shakes, Scratching, Grooming, Chewing, Seizure, Eating, Wet Dog Shakes, Drinking, Locomotion and Immobility, Freezing, Startle response). We consistently develop new behavior detection software for Laboras. There are over 1000 publications about the use of Laboras by several leading researchers, pharmaceutical companies, CRO's and leading universities from around the world. You may access these also from our website.

SONOTRACK: Completely automatic recording, playback and visualization of ultrasounds vocalizations in laboratory animals (15KHz-125Khz).

Sonotrack is an advanced system to record, analyze and playback ultrasound vocalizations. The system is highly valued for research in Anxiety, Stress, PTSD, Memory, Learning, Pain, Sexual related, Safety Pharmacology, Developmental (Neuro) Toxicity and Social Interaction tests. Sonotrack is the best ultrasound vocalization system on the market today because of its full spectrum USV recording (15 KHz to 125 KHz) characteristics, extremely low noise, long duration recording capability and reliable detection of long rodent calls fully automatically!!

Sonotrack enables you to record multiple animals simultaneously. The System has 4 channels (microphones), which enables researchers to record 4 independent environment/cages simultaneously. There can be more than 1 animal in each cage. In such a situation, multiple animals will be recorded at each channel. This is extremely beneficial in tests involving mother-pup interaction, male-female interaction etc.

A consistent/standardized environment is essential to build reliable behavioral study and analysis.

SmartChamber: SmartChamber is an advanced sound attenuation chamber with integrated video capability, light/dark setting and ventilation (monitoring video and control of the internal conditions via tablet). The SmartChamber ensures a standardized environment especially critical for USV experiments. It improves the ultrasonic recordings by creating a measurement environment that eliminates most of the environmental influences, echo's, other disturbances etc. from the laboratory environment. It ensures a stable and more controlled experimental environment.

Conclusion

Current trends in the Pharmaceutical industry require new translational approaches for pre-clinical test. Those aspects can be achieved by animal experiments in which not only one variable (e.g. one behavior) at the time is analyzed but rather a multidimensional approach (physiology +several behaviors + Ultra Sounds Vocalization + different parameters from study) is applied. Therefore, automation and integration of different measuring technologies become the crucial aspects in this process. The behavioral tests that are used in the ASD animal models studies, are not always of procedural standard.

Furthermore, contradictive results have been found. Standardizing procedures is important in this field of research. However, these experiments should be designed in a way that the animal is minimally restricted in performing its natural behavior, therefore Stress-free collection of continuous rodent behaviors and constant environmental conditions are crucial.

Currently, there is lack of a gold standard in behavioral phenotyping research. This is illustrated by the observation that many behavioral tests are used to measure the same parameter on basis of different definitions. In the case of ASD, stereotypies are most often measured in duration of self-grooming, although some studies think of a different way to quantify this behavior (duration or frequency in time bins). Furthermore, the fact that a different parameter is used here, makes it difficult to compare the results of this experiment to quantifications of Grooming behavior in other studies. For USV vocalization study there is no standard measuring parameters, often not standard and constant environmental conditions and not standard ultrasounds call classification model. By critically reviewing and investigating all USV rodents call models that are currently used, Metris BV has developed definitions and powerful algorithm: Sonotrack automated ultrasonic call classification for mice.

Validated gold standard phenotyping testes for quantifying behavioral parameters is essential for behavioral research.

We have proceeded far along the road towards an ideal measuring method of ASD with Laboras and Sonotrack system. By critically reviewing all ASD animal models that are currently used, and the behavioral phenotyping assays that are applied, future directions are pointed out. Animal models are always a compromise between mimicking the complexity of the actual disorder and designing practical experiments. Combining knowledge, we narrow down the funnel towards not only a gold standard model, but also a gold standard behavioral phenotyping strategy, thereby increasing the translational capacity of the animal model.

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