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Revisão Sistemática

Correlations between stereotypies in ASD and neurotransmitters: a systematic review

Correlações entre estereotipias no TEA e neurotransmissores: revisão sistemática

Correlaciones entre estereotipias en TEA y neurotransmisores: una revisión sistemática

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Resumo

Introdução. Há evidências que neurotransmissores e seus sistemas podem estar envolvidos na fisiopatologia de estereotipias motoras no Transtorno do Espectro Autista. **Objetivo.** Conduzir uma revisão para avaliar evidências sobre correlações entre comportamentos estereotipados e alterações nos sistemas de neurotransmissores, pela análise de artigos originais em modelo animal. **Método.** Estudos publicados entre janeiro de 2000 e janeiro de 2021 foram pesquisados em seis bases de dados e 19 estudos foram selecionados. A população, o método, os principais resultados e as conclusões dos artigos foram extraídos. **Resultados.** A análise revelou que os sistemas serotoninérgico, dopaminérgico, glutamatérgico, colinérgico e GABAérgico estavam envolvidos na modulação de estereotipias nos modelos animais do TEA. Em geral, a administração de antagonistas de receptors serotoninérgicos, glutamatérgicos e dopaminérgicos causaram uma redução no comportamento estereotipado, e a estimulação dos sistemas GABAérgico e colinérgico, através da administração de agonistas de receptors, também foram responsáveis por reduzir esses comportamentos. Houve algumas discrepâncias nas respostas dependendo das rotas de administração da droga e das áreas cerebrais, sugerindo que esses fatores também influenciam na fisiopatologia das estereotipias no TEA. **Conclusão.** Os estudos sugerem que a super-ativação dos sistemas serotoninérgico, glutamatérgico e dopaminérgico, assim como a supressão dos sistemas GABAérgico e colinérgico podem ter uma influência direta no aparecimento e na modulação de estereotipias. Registro: PROSPERO CRD42021235397

Unitermos. Comportamento Estereotipado; Transtorno do Espectro Autista; Neurotransmissores; Modelos Animais

Abstract

Introduction. There is evidence that neurotransmitters and their systems may be involved in the pathophysiology of motor stereotypies in Autism Spectrum Disorder. **Objective.** Conduct a review to assess evidence on correlations between stereotyped behaviors and changes in neurotransmitter systems, by analyzing original articles in an animal model. **Method**. Studies published between January 2000 and January 2021 were searched in six databases and 19 reports were selected. Reports' population, method, main results, and conclusions were extracted. **Results.** The analysis revealed that serotonergic, dopaminergic, glutamatergic,

cholinergic, and GABAergic systems were involved in the modulation of stereotypies in animal models of ASD. In general, the administration of antagonists of serotoninergic, glutamatergic, and dopaminergic receptors causes a reduction in stereotyped behavior, and the stimulation of GABAergic and cholinergic systems, through the administration of receptor agonists, was also responsible for reducing these behaviors. There were some discrepancies in the responses depending on the routes of drug administration and the brain areas, suggesting that these factors also influence the pathophysiology of stereotypies in ASD. **Conclusion**. The reports suggest that the over-activation of serotoninergic, glutamatergic, and dopaminergic systems, as the suppression of GABAergic and cholinergic systems may have a direct influence on the appearance and modulation of stereotypies. Registration: PROSPERO CRD42021235397.
Keywords. Stereotyped Behavior; Autism Spectrum Disorder; Neurotransmitters; Animal Models

Resumen

Introducción. Existe evidencia de que los neurotransmisores y sus sistemas pueden estar involucrados en la fisiopatología de las estereotipias motoras en el Trastorno del Espectro Autista. **Objetivo**. Realice una revisión para evaluar la evidencia sobre las correlaciones entre los comportamientos estereotipados y los cambios en los sistemas de neurotransmisores, mediante el análisis de artículos originales en un modelo animal. **Método**. Se buscaron estudios publicados entre enero de 2000 y enero de 2021 en seis bases de datos y se seleccionaron 19 estudios. Se extrajo la población, el método, los principales resultados y las conclusiones de los artículos. **Resultados.** El análisis reveló que los sistemas serotoninérgico, dopaminérgico, glutamatérgico, colinérgico y GABAérgico estaban involucrados en la modulación de estereotipias en modelos animales de TEA. En general, la administración de antagonistas de los receptores serotoninérgicos, glutamatérgicos y dopaminérgicos provocó una reducción de las conductas estereotipadas, y la estimulación de los sistemas GABAérgico y colinérgico, mediante la administración de agonistas de los receptores, también fueron responsables de la reducción de estas conductas. Hubo algunas discrepancias en las respuestas según las vías de administración del fármaco y las áreas del cerebro, lo que sugiere que estos factores también influyen en la fisiopatología de las estereotipias en los TEA. **Conclusión.** Los estudios sugieren que la sobre activación de los sistemas serotoninérgico, glutamatérgico y dopaminérgico, así como la supresión de los sistemas GABAérgico y colinérgico, pueden tener una influencia directa en la aparición y modulación de estereotipias. Registro: PROSPERO CRD42021235397
Palabras clave. Comportamiento Estereotipado; Desorden del espectro autista; neurotransmisores; Modelos de animales

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in communication and social interaction and by restricted, repetitive/stereotyped behaviors. Motor disorders such as simple motor stereotypies and repetitive use of objects are

among the most common repetitive behaviors and are present in a large proportion of autistic individuals[1,2].

Motor stereotypies are generally rhythmic and repetitive movements that can manifest in different parts of the body, such as hands, legs, arms, and face, and are usually suppressed by distraction[3]. Motor stereotypies can include snapping the fingers, swinging arms or legs, and arranging objects in a certain order, among others[1,3]. When related to some coexisting disorders, such as ASD, motor stereotypies are classified as secondary, that is, non-physiological[3]. Motor stereotypies can establish severity levels of ASD, and when these manifestations are severe, they can significantly reduce the individual's quality of life[1].

Although they are common manifestations in ASD, motor stereotypies do not yet have well-defined pathophysiology. However, there is evidence that brain neurotransmission may play an important role in the appearance and modulation of motor stereotypies[2,4]. Several studies have reported the relevance of the dopaminergic system in modulating stereotypies in ASD, mainly because dopamine has a great influence on brain motor pathways[5]. However, the pathophysiology of stereotypies seems to involve several neurotransmission systems and mechanisms that are not yet fully elucidated, as well as specific brain areas, which makes it difficult to understand the etiology of symptoms[2].

Serotonin (5-HT), for example, is a neurotransmitter associated with ASD for many years. Schein and Freedman

found that children diagnosed with ASD had elevated levels of serotonin in their blood, suggesting a link between this neurotransmitter and ASD[6]. In addition, glutamate and GABA are responsible for the excitation-inhibition balance in the central nervous system, which is why it has been hypothesized that disruption of this balance is associated with the occurrence of various neurodegenerative and psychiatric disorders[7-10].

As an important neurotransmitter, acetylcholine is closely linked to CNS development. In addition to regulating certain behaviors expressed in response to environmental stimuli, it may also be associated with the presence of stereotypies[11,12]. In addition, we highlight the role of dopamine as a neurotransmitter that directly contributes to the regulation of movements associated with certain motor disorders[13].

The pathophysiology of stereotyped behaviors in ASD may be multifactorial and intrinsically related to the neurotransmitters mentioned above. Understanding the pathophysiology of motor stereotypies is essential for the development of pharmacological and non-pharmacological treatments that aim to suppress stereotyped behaviors, and thereby improve the quality of life of individuals who have such symptoms.

Thus, the present study was conducted to evaluate the evidences between the association of stereotyped behaviors and changes in neurotransmitter systems, through the analysis of original articles carried out in animal model.

METHOD

Source of Information and Research Strategy

This Systematic Review was registered in the International Prospective Register of Systematic Reviews (PROSPERO CRD42021235397). The literature search covered January 2000 to October 2022. The databases used were: MEDLINE, LILACS, Scopus, SciELO, ScienceDirect, and EMBASE. The research strategy that we used is: "Autism Spectrum Disorder", "Autistic Disorder", "Stereotyped Behavior", "Stereotypic Movement Disorder", and "Neurotransmitter Agents". Was used the descriptors, Mesh, EMTREE, and DeCS to the databases MEDLINE, EMBASE, and LILACS, respectively. The data analysis started in October 2022 and finished in December 2022.

Eligibility Criteria

Inclusion criteria

Articles that showed an association between increased or decreased activity of neurotransmitters in the central nervous system and manifestations of stereotyped motor behaviours were selected. Only original articles written in English, Spanish, or Portuguese and published from January 2000 to October 2022 were considered.

In vivo or *ex vivo* animal studies (mice and rats) of any age and both sexes receiving agonists or antagonists of neurotransmitters (serotonin, dopamine, glutamate, GABA,

norepinephrine, and acetylcholine), and who demonstrate stereotyped motor behaviours. There was no delimitation of doses and duration of treatment in the study protocols.

Exclusion Criteria

Articles were in the scope of the research question. Articles with insufficient data for analysis. Studies in which the methodology was insufficient to associate neurotransmitter activity and stereotypes. Publications in the form of reviews, letters, editorials, and comments. Human, *in vitro*, and *in silico* studies were excluded. Models of other species that are not in the inclusion criteria. Articles that did not have control groups and pilot studies were excluded.

Risk Quality Assessment and Bias Report

The authors conducted a risk of bias analysis using the OHAT Risk of Bias Rating Tool for Human and Animal Studies scale. This scale was constructed to be an objective way of analyzing bias in studies for humans and animals, eliminating potential threats to the validity of the study[14].

The scale aims to assess the risk of bias both concerning the methodology and the results of each study and is divided into 6 sections: Selection bias, Performance bias, Attriction/Exclusion bias, Detection bias, Selective Reporting bias and Other bias. The scale has 11 questions applied to studies with animals (unlike studies with humans, which are 13 questions). Each response can be graded for risk of bias into "definitely high", "probably high", "probably low" and

"definitely low"[14]. The scale was applied to all manuscripts chosen to compose the present study.

The quality assessment of the studies was carried out using the Quality Assessment of Controlled Intervention Study tool, a scale developed by the NHLBI in 2013. The scale was initially developed for studies with humans, needing to be adapted for the present study in animals, thus some scale questions were not applicable to the analyzed manuscripts[15].

The scale focuses on the internal validity of the studies and addresses, among other questions, whether the study is described as randomized, whether the allocation was randomized and blinded, whether the study was blinded, whether the groups are similar in baseline characteristics, whether there was loss of participants of the study, whether the participants adhered to the protocols of the study interventions, whether other types of intervention were avoided, whether there was a good evaluation of the outcome measures, whether the confidence interval was satisfactory, whether the results were pre-specified, and finally if all randomized participants were analyzed in the groups that were originally placed[15].

Strategy for Data Synthesis and Extraction

Primarily, two independent reviewers searched the literature using the descriptors previously mentioned. Then, the duplicates were removed. After, the reviewers assessed all remaining titles and abstracts for eligibility. The full-text

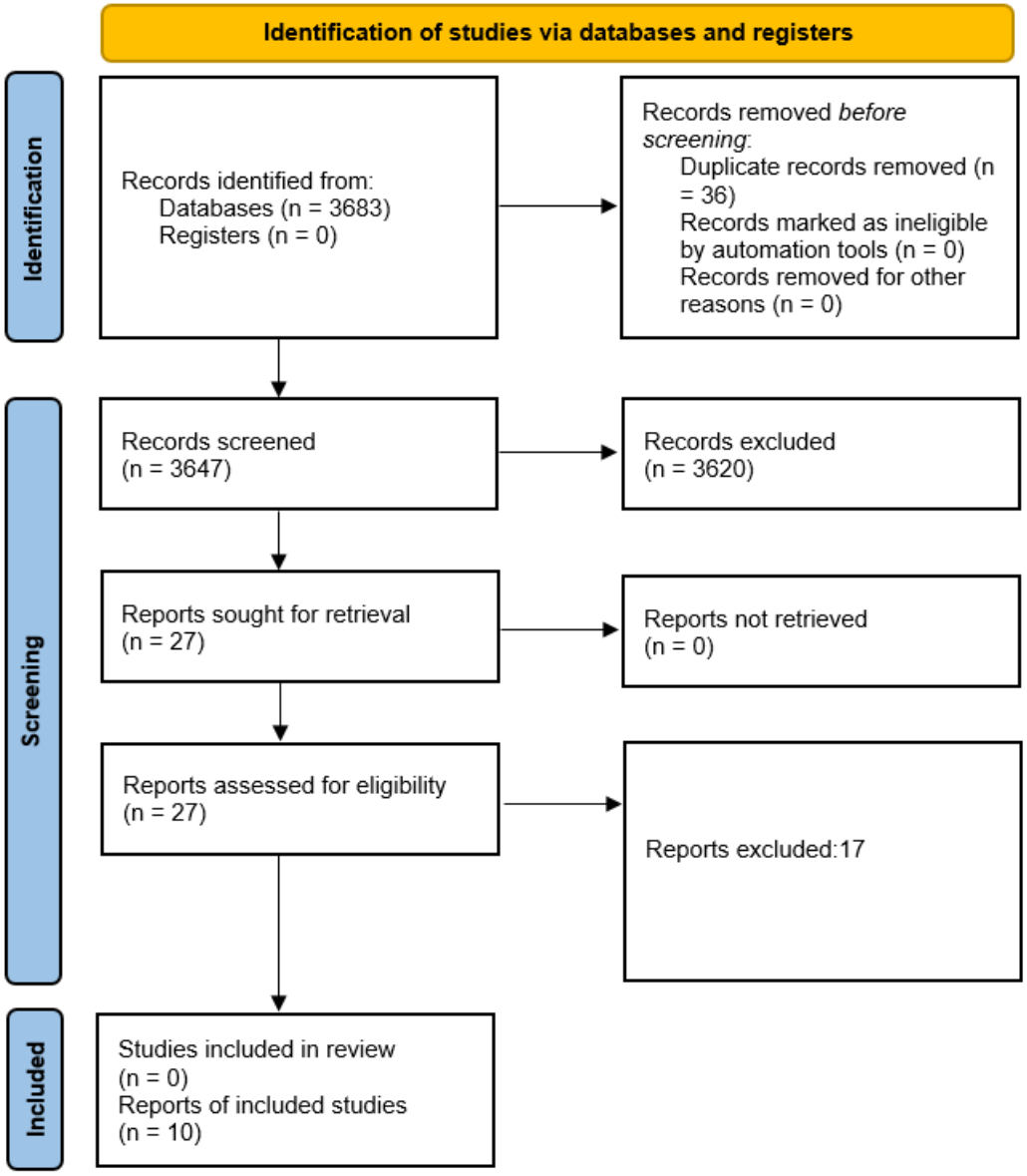
articles that were insufficient for determining eligibility were retrieved. The full text of the remaining articles that matched the inclusion criteria was analysed separately by these two reviewers. Due to disagreement between them about the eligibility of certain studies, a third reviewer decided if certain studies were included or not. Two independent reviewers performed the data extraction and research of the literature, using their knowledge of the subject of this review and inclusion criteria to analyse the results. The eligibility of the articles was judged independently by two groups with one reviewer. Each reviewer of each group screened the reference lists of all the included articles for any additional relevant studies. There were team meetings in each review phase to discuss criteria. Many abstracts and articles were pilot tested to ensure agreement. Discussion and consensus resolved disagreements among reviewers. There was no need to contact the study's authors to identify additional or missing information yet to be published or lost.

RESULTS

A total of 3683 papers were found, after the inclusion and exclusion filters, in the six databases searched: Medline, Lilacs, SCOPUS, EMBASE, ScienceDirect, Web Of Science and Scielo. After this step, duplicates were excluded, so 36 papers were manually removed. Of the remaining 3647 articles, 3620 were excluded for not meeting the inclusion or exclusion criteria or the objectives of this review, resulting in 27 articles for full analysis. Seventeen articles were also

excluded because they did not meet the randomization criteria. Ten articles met the criteria for this paper and were included in the final review. The flow chart of study selection is shown in Figure 1.

Figure 1. Flow chart of study selection.



All studies selected for this review were conducted with animals, either mice or rats. Among included papers, nine (90%) used mice and one (10%) used rats. Among mice, the most prevalent type for simulating stereotypic behaviors typical of Autism Spectrum Disorder was BTBR mice, so four studies (40%) evaluated this species. As for the behaviors that simulate stereotypies, the one with the highest prevalence was the grooming behavior and marble-burying, evaluated in five studies each (50%). Of the included studies, six (60%) evaluated the GABAergic system. The glutamatergic system was analyzed in one study (10%), another two (20%) analyse the serotoninergic system, and the dopaminergic system was studied by one paper (10%). Table 1 summarizes the main findings of this review.

Table 1. Summary of the studies included in this review including the murine models used, behaviors evaluated, and neurotransmitters and receptors studied.

Authors	Neurotransmitter	Repetitive behavior	Receptors
Amodeo 2017[16]	Serotonin	Grooming	$5TH_{2A}$
Canal 2015[17]	Serotonin	Idiopathic stereotypic jumping; head-twitching; stereotypic rotations	5-HT_7 and 5-HT_{1A}
Mohammadi 2019[18]	Glutamate	Self-grooming; rearing	NMDA
Jiang 2022[19]	GABA	Marble-burying	$GABA_B$
Jiang 2022[20]	GABA	Marble-burying	$GABA_B$
Silverman 2015[21]	GABA	Self-grooming; stereotyped jumping	$GABA_B$
Venkatachalam 2021[22]	Dopamine	Marble-burying	D2R/D3R
Yang 2021[23]	GABA	Marble-burying	$GABA_A$
Yang 202124	GABA	Self-grooming; Marble-Burying	$GABA_A$; $GABA_B$
Yoshimura 2017[25]	GABA and Glutamate	Self-grooming	$GABA_A$, mGluR5

Bias analysis of the articles, performed using the OHAT scale, showed the following results: Regarding the

randomization of doses, 40% of the articles obtained a classification of definitely low risk of bias and 60% obtained a probable low risk of bias. As for allocation randomization, 80% of the articles were classified as definitely low risk, and 20% as likely low risk. It was also seen that in 90% of the articles, there was evidence that the conditions experienced by the animals were the same, definitely configuring a low risk of bias, and the other 10% showed a probable low risk. As for the research group being blinded to the study, 60% of the articles showed a probable low risk of bias, and 40% definitely showed low risk. In addition, 50% of the studies reported complete data without wear or exclusion from the analysis, definitely configuring a low risk of bias, while the other 50% denoted a likely low risk. All studies had a satisfactory confidence interval for the characterization of exposure (100% definitely low risk of bias). As for the evaluation of the results, 90% had significant confidence intervals ($P<0.05$), being considered as definitely low risk of bias, and 10% reported variable confidence intervals, that is, probable risk of bias. Furthermore, 100% of the studies reported all the results found. Finally, 80% of the studies definitely obtained a low risk of bias because no other potential threats of bias were identified, and the other 20% obtained a probable low risk of bias because they presented some potential threat of bias, such as a conflict of interest of the authors. The results are shown in Table 2.

Table 2. Risk of bias assessment based on OHAT tool.

	Selection bias		Performance bias		Attrition/exclusion bias		Detection bias	Selective reporting bias	Other bias
Question	**1**	**2**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Amodeo 2017[16]	+	+	+	+	+	++	++	++	+
Canal 2015[17]	++	++	++	++	+	++	+	++	++
Mohammadi 2019[18]	++	++	++	+	++	++	++	++	++
Jiang 2022[19]	+	++	++	+	+	++	++	++	++
Jiang 2022[20]	+	++	++	++	++	++	++	++	++
Silverman 2015[21]	++	+	++	++	++	++	++	++	++
Venkatachalam 2021[22]	+	++	++	+	++	++	++	++	++
Yang 2021[23]	+	++	++	+	+	++	++	++	++
Yang 2021[24]	+	++	++	+	++	++	++	++	++
Yoshimura 2017[25]	++	++	++	++	+	++	++	++	+

Questions are grouped under 6 types of bias (selection, performance, attrition/exclusion, detection, selective reporting, and other bias). The selected question is applicable to experimental animal. Questions are rated by selecting among 4 possible answers. Definitely Low risk of bias (++); Probably Low risk of bias (+); Probably High risk of bias (-); Definitely High risk of bias (--). Question 1: Was administered dose or exposure level adequately randomized?; Question 2: Was allocation to study groups adequately concealed?; Question 5: Were experimental conditions identical across study groups?; Question 6: Were the research personnel and human subjects blinded to the study group during the study?; Question 7: Were outcome data complete without attrition or exclusion from analysis?; Question 8: Can we be confident in the exposure characterization?; Question 9: Can we be confident in the outcome assessment?; Question 10: Were all measured outcomes reported?; Question 11: Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?.

The analysis of the quality of the studies, using the Quality Assessment of Controlled Intervention Study tool, showed: 100% of the studies were randomized, with appropriately randomized allocation. In addition, all animals participating in the studies have similar baseline characteristics. The proposed protocols were followed in all studies, and other interventions were avoided in order to avoid doubtful results. The results reported in the studies had been pre-specified and had a significant confidence interval ($p<0.05$). The dropout issue does not apply to mouse studies. Furthermore, 50% of the studies stated that research personnel were blind to the results and methods, while the other 50% reported no such result. Table 3 summarizes the findings of each article.

Table 3. Quality assessment of controlled intervention study tool.

Question	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Amodeo 2017[16]	Yes	Yes	Yes	NR	NR	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Canal 2015[17]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Mohammadi 2019[18]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Jiang 2022[19]	Yes	Yes	Yes	NR	NR	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Jiang 2022[20]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Silverman 2015[21]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Venkatachalam 2021[22]	Yes	Yes	Yes	NR	NR	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Yang 2021[23]	Yes	Yes	Yes	NR	NR	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Yang 2021[24]	Yes	Yes	Yes	NR	NR	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
Yoshimura 2017[25]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes

There are 14 questions with 3 possible answers: Yes, No and Other (CD, cannot determine; NA, not applicable; NR, not reported). Question 1: Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT?. Question 2: Was the method of randomization adequate (i.e., use of randomly generated assignment)?. Question 3: Was the treatment allocation concealed (so that assignments could not be predicted)?. Question 4: Were study participants and providers blinded to treatment group assignment?. Question 5: Were the people assessing the outcomes blinded to the participants' group assignments?. Question 6: Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)?. Question 7: Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment?. Question 8: Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower?. Question 9: Was there high adherence to the intervention protocols for each treatment group?. Question 10: Were other interventions avoided or similar in the groups (e.g., similar background treatments)?. Question 11: Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?. Question 12: Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power?. Question 13: Were outcomes reported or subgroups analyzed prespecified (i.e., identified before analyses were conducted)?. Question 14: Were all randomized participants analyzed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis?.

DISCUSSION

The discussion of this work was based on the 10 selected articles that showed a good performance on the OHAT scale, thus, it is possible to state that the articles included and discussed in the present study were randomized and presented a low risk of bias, therefore being reliable and pertinent to the theme presented in this study. The quality analysis of the studies also confirms that the selected articles have good quality and internal validity, sufficient to extract important translational information about the ASD.

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a substance derived from 5-hydroxytryptophan that plays a

neurotransmitter role[26,27] and has long been correlated with ASD. In 1961, Schain and Freedman found extremely high levels of serotonin in the blood of six of 23 children with ASD, i.e., about 26% of the study participants[6]. These findings suggested that serotonin may have a relevant role in the pathophysiology of ASD and allowed the emergence of a new field of research relating to serotonin and ASD. The receptors of the serotoninergic system that appear to be involved in the pathophysiology of stereotypies in ASD are $5HT_{2A}$, $5\text{-}HT_{6}$, $5\text{-}HT_{1A}$, and $5\text{-}HT_{7}$[16,17,28-30].

The serotonin 2A receptor ($5HT_{2A}$) has multiple functions in the central nervous system (CNS), being present in brain areas responsible for cognition and social interaction. This receptor may be involved in the pathophysiology of several mental disorders such as schizophrenia, obsessive-compulsive disorder, and attention deficit hyperactivity disorder[31].

In the stereotypies present in ASD, a study by Amadeo 2016[16] showed that, from the infusion of M100907, a $5HT_{2A}$ antagonist, into the dorsomedial striatum, a brain region often correlated with ASD and repetitive behaviors, there was a reduction of self-grooming in a mouse model exhibiting this behavior. A similar result was found in another study using the same agonist[28], suggesting that activation of the serotoninergic system may be involved with the onset of stereotypic behaviors. Intraperitoneal administration of risperidone, which exerts blockade on dopaminergic D2 and serotoninergic $5\text{-}HT_{2A}$ receptors, was also effective in

reducing stereotypies in mice at sedative doses, reinforcing the hypothesis that the 5-HT_{2A} receptor is involved in the pathophysiology of stereotypic behaviors in ASD[30].

However, Amodeo 2016[16] also demonstrated that infusion of the $5HT_{2A}$ antagonist, M100907, into the orbitofrontal cortex promoted increased stereotypies. The orbitofrontal cortex (OFC) is a brain area also associated with ASD and seems to correlate, mainly, with the communication deficits present in autistic individual[32]. However, the results demonstrated by Amodeo 2016[16] indicate that the OFC may also be related to the modulation of repetitive behaviors. Thus, it is possible to assume that the pathophysiology of stereotypies in ASD is multifactorial, and, therefore, new pharmacological treatments for stereotypies must have specific targets and locations, since the inhibition of the $5HT_{2A}$ receptor in the dorsomedial striatum and OFC in ASD models caused opposite behaviors.

The 5-HT_6 receptor also seems to be related to the manifestation of stereotypes in ASD[29]. Discovered in the 1990s, the 5-HT_6 receptor has a wide distribution in the central nervous system, being present in abundance in the striatum, as well as in the nucleus accumbens and cerebral cortex[33]. This receptor also appears to influence an individual's learning, cognition, and affective state, through the regulation of cholinergic and glutamatergic neuronal activities and which may be compromised in ASD[1,33]. By intraperitoneal administration of a 5-HT_6 receptor antagonist (BCG 20-761 or Tocris) in mice with stereotypies, had a

significant reduction in this behavior occurred. This result also suggests that decreasing the activity of this receptor may reduce stereotypic behavior.

Another serotonin receptor potentially involved in the pathophysiology of stereotypies is 5-HT_{1A}. This receptor is widely expressed in several brain areas such as the thalamus, hypothalamus, and brainstem nuclei, among others, and may be involved in the pathophysiology of different mental disorders, such as ASD[34]. Playing an important role in regulating 5-HT release in the brain, it has been suggested that activation of this receptor can treat catalepsy and dyskinesia due to the suppression of striatal dopaminergic neurons, caused by serotoninergic action[17].

The 5-HT_7 receptor is found in several brain areas but is highly expressed in the hypothalamus, and its regulation is associated with the pathophysiology of several disorders, including sleep disorders, unipolar depression, learning and memory problems, anxiety, and other disorders that are related to ASD[35,36]. It is also known that 5-HT_7 receptors are also abundant in the thalamus, an important neural system in the regulation of motor behavior[37].

Indeed, 5-HT_{1A} and 5-HT_7 receptors appear to be involved in the manifestation of stereotypic behavior. By administering a partial agonist of these receptors, Canal 2015[17] observed complete elimination or attenuation of idiopathic stereotypic repetitive behaviors of jumping, head-twitching, and stereotypic rotations, indicating that such

receptors may be involved in the negative modulation of stereotypies.

Therefore, different receptors of the serotoninergic system may play opposing roles concerning the modulation of stereotypic behaviors, possibly being influenced by other receptors and the site of activation.

Dopamine

Dopamine is a monoaminergic neurotransmitter, such as serotonin and norepinephrine, and contributes to the regulation of movements[38,39]. For this reason, dopamine is associated with several motor disorders, such as Parkinson's disease, which have pharmacological treatment based on dopamine[13]. Dopamine also appears to be involved in other neuropsychiatric disorders such as schizophrenia and obsessive-compulsive disorder[40,41]. Studies on the action of dopamine in ASD and the pathophysiology of stereotypes have been gaining importance since dopamine plays an important role mainly in striatal pathways, which correlate with motor control[32,42-44].

Dopaminergic D3 receptors (D3R) are important targets of antipsychotic drugs in the mesolimbic system and are related to the production of extrapyramidal symptoms. High expression of DRD3 receptor binding sites are found in the limbic striatum (nucleus accumbens and putamen), and in humans, D3R receptors are also highly expressed in the motor striatum. Coexpression with the D2 receptor occurs in many brain regions, including the thalamic nuclei and the

amygdala. In ASD, there are alterations in the basal ganglia in particular in the caudate nucleus. Repetitive and stereotyped behaviors may be associated with abnormalities present in the corpus striatum of autistic individuals. These changes may be related to polymorphisms in DRD3 gene[45].

Dopaminergic receptors, in general, share characteristics regarding their structure. D2 and D3 receptors, for example, have expressive similarities (75%) about their transmembrane domains. These two receptors, together with the D1 receptor, seem to control the locomotor activity of the individual, acting in the ventral striatum. The D2 receptor has a synergistic action with the D1 receptor in reducing and increasing locomotor activity, respectively, while the D3 receptor seems to inhibit this activity, so a D3R antagonist stimulates motor activation[46]. Therefore, altering the activity of these receptors may affect the individual's motricity, and may play a significant role in modulating stereotypes.

In this context, since D2R and D3R have an inhibitory action on locomotor activity, it would be possible to assume that the activation of these receptors could reduce the frequency or duration of stereotypic behavior. However, Venkatachalam 2021[22] saw that chronic intraperitoneal administration of a dopamine and histamine receptor antagonist, respectively D2R/D3R and H3R, reduced the number of marble-burying in mice exhibiting such behaviors. In addition, another study saw that intraperitoneal treatment with risperidone in sedative doses affected reducing

stereotypies in mice[30]. Risperidone targets the D2 receptor, being an inverse agonist, i.e., having an antagonistic effect[30,47]. Thus, this study is also suggestive that increased dopamine release, through D2R inhibition, would be compatible with increased stereotypic behaviors.

These results confirm the assumption of the existence of a relationship between increased dopaminergic release and the incidence of stereotypies. Despite the results found, the release of dopamine seems to be influenced by other neurotransmitters. For example, the H_3 receptor acts as a postsynaptic heteroreceptor, that is, it is present in non-histaminergic neurons, and therefore acts by regulating the release of other neurotransmitters, such as dopamine, serotonin, glutamate, GABA, and acetylcholine, which may justify the contradiction found[48,49].

Furthermore, the regulation of dopamine homeostasis relies on the dopamine transporter (DAT), a presynaptic transmembrane protein that regulates dopaminergic tone in the central nervous system through high-affinity reuptake by dopamine released into the synaptic cleft. Evidence suggests that DAT dysfunction leads to alterations in dopaminergic signaling, and this leads to changes in the pattern of behaviors associated with neuropsychiatric disorders, such as ASD. Mutations in this transporter (DAT) producing abnormal functions, characterized as a persistent reverse transport of DA (substrate efflux) have been linked to ASD. In an experiment using Drosophila melanogaster, expression of hDAT T356M in DA neurons-lacking Drosophila DAT led to

increased hyperlocomotion, a characteristic associated with dopaminergic changes in autism[50].

In a study using a genetically modified knockin mouse model in which threonine-methionine at site 356 (DAT T356M), a mutation identified in individuals with ASD, was replaced a reduction in abnormal DA reuptake and efflux was observed. The authors of this study concluded that excess DA in the synaptic space would result in desensitization of D2R receptors, reduced DA synthesis, and reduced total tissue DA content[51].

GABA

GABA (γ-aminobutyric acid) is an inhibitory neurotransmitter related to several psychiatric disorders. This neurotransmitter, together with glutamate, plays an important role in controlling the excitation/inhibition balance, so that a decrease or increase in the activity of one of the two main neurotransmitters responsible for this balance may promote important organic repercussions[9,10].

The GABAergic system is responsible for many of the synapses in the CNS and has two types of receptors, $GABA_A$ and $GABA_B$[10,52]. Activation of $GABA_A$ receptors, an ionotropic receptor, promotes inhibition of neuronal membrane excitability usually by cellular hyperpolarization, through control of chloride flux in the cell[52]. Thus, the decreased functioning of GABAergic receptors would allow cell depolarization and thus the predominance of excitatory activity.

Alterations in the GABAergic signaling pathway are responsible for autism-like stereotypes in most animal models of ASD from experimental manipulation of candidate genes or environmental risk factors for autism susceptibility. The phenotypic features linked to ASDs are often associated with positive or negative regulation of GABAergic function. Alterations in GABA synthesizing enzymes such as GAD65 and GAD67, variations in GABA release, and expression of particular $GABA_A$ receptor subtypes have been detected in patients with ASD[53].

Stereotyped movements, compulsive cleaning, impaired motor coordination, learning/memory deficits may be related to the deficiency of the MeCP2 protein in GABAergic neurons. This deficiency in a subset of GABAergic neurons in the prosencephalon also impairs motor coordination, causes repetitive behaviors, and alters control of sensory-motor activation and arousal, thus loss of MeCP2 in GABAergic neurons, either globally or in a subset, signals for an abundance of neuropsychiatric phenotypes[54].

Concerning ASD, it has been seen that $GABA_A$ receptor agonists, as well as the action of positive allosteric modulators, reduce several stereotypic behaviors, such as grooming, marble burying, and head movements, in experiments using mice with autistic phenotypes[23,25,55,56].

In this context, Yang 2021[23] demonstrated that from the administration of clonazepam, considered a $GABA_A$ receptor agonist, in mice with autistic behaviors, there was attenuation of the evaluated behavior (marble-burying), with

a reduction in GABAergic neurotransmission also being evidenced in the medial prefrontal cortex of the offspring.

Likewise, the inhibition of the $GABA_A$ receptor, through the administration of the antagonist picrotoxin, demonstrated an increase in self-grooming[57]. These results are consistent with the idea that stereotypes may be associated with increased excitatory activity in the CNS.

Metabotropic $GABA_B$ receptors also promote cellular inhibition. Their inhibitory mechanism happens through the opening of potassium channels and the inhibition of calcium channels, promoting neuronal hyperpolarization[52]. Regarding stereotypes, similar to what was seen with $GABA_A$ receptors, Silverman 2015[21] showed that intraperitoneal administration of selective $GABA_B$ receptor agonist R-baclofen and Baclofen strongly reduced stereotypic and repetitive behaviors in two mouse strains (BTBR and C58), such as self-grooming, high marble burying, and high vertical jumping. In addition to reducing stereotypies, this GABAergic agonist was shown to be able to reduce anxiety behaviors, therefore improving sociability deficits in mice exposed to valproic acid (VPA), as demonstrated by Jiang 2022[19] and Jiang 2022[20].

Thus, the pathophysiology of stereotypies may have a common path with the pathophysiology of the social behavior of the individual with autism, so that future treatments based on this neurotransmitter could improve all the central symptoms of ASD. Thus, new experimental studies focused on GABA agonism may demonstrate a great improvement in

the issue of sociability and motor behavior of these individuals.

Confirming the results, a study that evaluated $GABA_A$ and $GABA_B$ receptor agonists demonstrated that the activation of these receptors can reverse the typical behaviors of autism in mice exposed to VPA. Furthermore, it was observed that these mice still have reduced GABAergic transmission, which supports the hypothesis of excitation/inhibition imbalance in individuals with ASD[24].

The results obtained are suggestive that reduced inhibition or increased neuronal excitability, i.e., loss of the excitation/inhibition balance represented by the neurotransmitters glutamate and GABA, result in increased excitability and may be associated with the onset of stereotypic behaviors in ASD. This also allows for the prospect of pharmacological therapy based not only on the antagonism of glutamatergic receptors but also on the agonism of GABAergic receptors to reduce these behaviors.

Yoshimura 2017[25] performed an experiment using several allosteric modulators in mice that exhibited autistic behaviors. The study concluded that the excitation/inhibition balance between glutamate and GABA could have a significant component in the pathophysiology of these behaviors. It was seen that the treatment of animals with autistic traits with a negative allosteric modulator of the mGluR5 receptor, was effective in reducing the time of self-grooming in relation to the control group. Furthermore, it

was seen that treatment with positive allosteric modulators of GABAA receptors also reduced self-grooming time.

One example with great potential for pharmacological therapy in ASD is bumetanide, which potentiates the action of γ-aminobutyric acid (GABA), altering the balance of synaptic excitation-inhibition, and decreasing the severity of ASD in animal models. Although the clinical evidence on the efficacy of this drug in children with ASD is still limited, a recently published clinical study shows positive results in decreasing the core symptoms of ASD, being associated with the reduction of the GABA/Glutamate ratio[58].

Thus, the results obtained suggest that the activation of the GABAergic system would be compatible with the reduction of stereotypic behaviors. In this context, since glutamatergic activation increases stereotypies and GABAergic activation reduces them, part of the pathophysiology of stereotypies may be explained by the loss of excitation-inhibition balance.

Concerning the excitation-inhibition (E-I) balance, it is necessary to understand that the quantity of excitatory and inhibitory synapses that occur in the central nervous system is kept in balance for the execution of a certain function. Thus, for a certain activity, with an increase in the number of excitatory synapses, there will be an increase in function, and with an increase in the number of inhibitory synapses, there will be a reduction in function. This balance is present at many levels, from neuronal to complex cortical circuits[59].

The neurotransmitters commonly related to the excitation-inhibition balance are glutamate and GABA, the former being responsible for the excitatory activity and the latter for the inhibitory activity, and there must be a coordination of stimuli between them to exist the balance[60,61].

In this context, alterations in normal development, such as mutations in genes that express GABAergic neurons and mutations in glutamatergic transporters, can impact this balance in a way that leads to the predominance of excitatory or inhibitory functions in certain areas of the CNS, which may be related to the onset of neuropsychiatric pathologies or disorders[59,61].

Based on this, E-I imbalance may be relevant in the pathophysiology of ASD, so there is evidence that primary defects in excitatory or inhibitory activity may trigger certain features of this disorder, especially related to social interaction deficits[59,60,62].

As for stereotypic behaviors, there are no significant amount of studies correlating stereotypies with E-I balance. However, a mouse experiment revealed that hypofunction of TRIO[63], a regulator of neuronal development, especially in the Guanine Nucleotide Exchange Factor 1 (GEF1) domain is related to both E-I imbalance, with decreased GABAergic activity, and behaviors typical of ASD[63,64]. In this study, they concluded that mice with TRIO dysfunction had increased stereotypic behaviors, as well as other behaviors related to

ASD, which were corrected from the activation of the GABAergic system[63].

Thus, it is possible to conclude that the loss of excitation/inhibition balance, represented by the neurotransmitters glutamate and GABA, may be associated with the onset of stereotyped behaviors in ASD. This allows for the prospect of a pharmacological therapy based on specific therapeutic targets related to excitation-inhibition balance. However, more studies are needed to elucidate the topic.

Glutamate

Glutamate is a molecule involved in excitatory neurotransmission and neuromodulation and is partially responsible for maintaining the excitation/inhibition balance in the CNS[7]. In the glutamatergic system, there are three groups of receptors: the metabotropic (mGluR), with several subtypes, and the ionotropic NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid). These receptor groups interact with each other so that glutamatergic synapses can be mediated by different combinations of receptors[8].

The glutamatergic system is closely related to neurotoxicity and is involved in the pathophysiology of several neurodegenerative diseases[8]. Specifically, about ASD, there is new evidence that changes in the excitation/inhibition balance, as well as in the glutamatergic

system in the CNS, may be part of the pathophysiology of this disorder[44,65-68].

The mGluR receptor subtypes act in the activation and inhibition of intracellular signaling, and this mechanism may be involved in the genesis of stereotypic behaviors[8]. Pharmacological activation of the mGluR5 receptor, by administration of agonists, intraperitoneally or brain stereotactically, produces stereotypic behaviors, while its suppression, observed by administration of the antagonist MPEP (a negative allosteric modulator), seems effective in reducing various types of stereotypies in animals with autistic behaviors[30,67,69,70].

The mGluR2/3 and mGluR1 receptors may also be involved in the pathophysiology of stereotypies in ASD. Suppression of mGluR2/3 by an antagonist promotes the onset of grooming and inhibition of marble-burying in WT mice, and administration of mGluR1 antagonist also has effects in reducing marble-burying in Eif4ebp2-/- mice[67,71]. These results may indicate that the pathophysiology of stereotypic behaviors may differ from each other, so there are possibly other factors involved that may influence the results, such as the animal species investigated and receptor interaction.

Ionotropic glutamatergic NMDA receptors, voltage-dependent receptors, are activated by AMPA-kainate receptors, which promote rapid responses, are voltage-independent, and are located next to NMDA receptors. When activated, the NMDA receptors act to further increase the

depolarization initiated by the AMPA receptors[8]. There is evidence that the NMDA receptor can also influence the onset of stereotypies[18,72,73].

From the administration of the NMDA receptor antagonist MK-801, Mohammadi 2019[18] showed a reduction in stereotypies such as grooming occurs in mice with typical ASD behaviors. Another study using memantine, an antagonist of the same receptor, was also effective in showing a reduction in jumping, another stereotyped behavior, which reinforces the theory that the activation of this receptor may be correlated to the emergence of stereotypic behaviors[72].

Furthermore, the direct injection of NMDA promoted an increase in self-grooming without affecting the mice's motor coordination. This result opens up the possibility that NMDA is a safe therapeutic target for individuals with ASD, as it can result in the improvement of stereotypies, without having motor side effects[74,75]. Already, the administration of D-cycloserine, a substance that acts as an NMDA receptor agonist at low doses, but an antagonist at high doses, improved the marble-burying behavior of mice exposed to valproic acid[73,76].

Thus, both metabotropic and ionotropic glutamatergic receptors appear to be involved in the pathophysiology of stereotypies in ASD. It is also possible that the interaction between the different receptors promotes different responses in the regulation of these behaviors. Despite the differences in results, in general, studies suggest that

increased activity of the glutamatergic may be correlated with the appearance of stereotypies in individuals with ASD, which allows a new line of pharmacological treatment of stereotypies targeting glutamatergic receptors in the striatum and hippocampus.

Acetylcholine

This neurotransmitter also seems to be associated with stereotypies in autism, but it was not addressed in the manuscripts selected for this work. In this way, we think it is important to bring some important information extracted from articles that did not fit any of the inclusion criteria, but we believe that it may provide a basis for a better understanding of stereotypies in ASD.

Acetylcholine is a brain excitatory neurotransmitter, which is involved in cell proliferation and regulation and the development of the central nervous system. This neurotransmitter is present in several regions of the CNS, such as the cerebral cortex, hippocampus, and brainstem[11]. In the central nervous system (CNS), acetylcholine (ACh) plays an important role in the response to environmental stimuli, responding according to their characteristics. Therefore, it performs the function of regulating relevant behaviors in people with ASD, in which attention, cognitive flexibility, social interactions, and stereotyped behaviors are included[12].

Many pieces of evidence associate the cholinergic system with ASD. As an example, we can cite that neurons

in a basal forebrain cholinergic nucleus of patients with ASD are unusual in number, size, and structure. In addition, low concentrations of choline, the precursor of ACh, have been found. It is estimated that low levels of cytosolic choline are associated with the severity of autism and that they may repercussion in a higher intensity of stereotypies[12].

Immunohistochemical evaluation of postmortem tissues revealed a reduction of several nAChR subunits and M1-type muscarinic-cholinergic receptors (mAChR) in the neocortex, cerebellum, thalamus, and corpus striatum of patients with ASD. One method of reaching the cholinergic system is through modulation of its receptors[77].

Muscarinic acetylcholine receptors (mAChRs), coupled to the G protein, have excitatory and inhibitory functions in the central nervous system, being part of postsynaptic excitation and pre and postsynaptic inhibition, even possessing mechanisms of self-inhibition[78]. There is evidence that these receptors can also modulate dopaminergic activity in some brain areas, such as the dorsal striatum, which has been associated with the development of stereotypes[19,79]. O

Oxytremonine (mAChR agonist) causes decreased self-grooming behavior in BTBR mice[80]. This indicates that activation of the cholinergic system is related to the suppression of stereotypic behaviors in individuals with ASD.

However, there is likely an interaction of acetylcholine with other neurotransmitters in the central nervous system, so the increase or suppression of stereotypies would occur due to several factors. In addition, it is possible that nicotinic

receptors of acetylcholine also exert influence on the onset of this behavior, as activation of brain neuronal components is also mediated by these receptors[79].

Based on the hypothesis that the increase in acetylcholine in the synaptic cleft caused by acetylcholinesterase inhibition would improve autistic phenotypes, a murine model study was conducted using donepezil, a centrally acting anticholinesterase and it was concluded that the elevation of ACh levels led to a significant improvement in cognition and social interactions, but failed to improve repetitive self-grooming and digging[12]. A possible explanation for this is because the onset to cease motor stereotypies depends on the balance between dopamine and cholinergic AChRs receptors in the striatum[12].

Prenatal exposure of rats and mice to valproic acid (VPA) in the model of autism induces dysregulation of cholinergic neuronal development, mainly the upregulation of acetylcholinesterase (AChE) in the prefrontal cortex. Sub chronic treatment with an anticholinesterase agent, donepezil, was able to improve sociability, as well as prevent repetitive behavior and hyperactivity in the offspring of mice induced ASD by VPA[81]. However further studies on the association between acetylcholine and stereotypies are needed to elucidate the issue.

Study limitations and future perspectives

As this work is a systematic review with studies carried out exclusively with animals (mice and rats), the study itself

already presents a translational bias regarding the species. Despite the similarity between the DNA of these animals and humans, the extrapolation of all results to the human species is not guaranteed.

It was also not possible to perform a meta-analysis of the results since the pattern of studies was heterogeneous. Furthermore, only manuscripts published in English, Spanish and Portuguese were included from the search, so it is possible that, although rare, some relevant work published in another language was not included.

Finally, this study managed to cover only five important neurotransmitters, which, although they have been widely associated with stereotypies, do not prevent other neurotransmitters not included or not detected by this study from being addressed. In this context, it is worth questioning whether other neurotransmitters not addressed in the study may affect these behaviors, in order to explain the discrepancies in some results of the study.

In this way, we emphasize the fact that in perspective for future works, it can be verified how neurotransmitters act depending on the brain area, that is, a clearer mapping of the role of neurotransmitters in the different brain areas. In addition, we can highlight that the molecular knowledge of the pathophysiology of ASD may positively impact the treatment of stereotypies, which is one of the core symptoms of autism and which does not find treatment available to date, therefore studies in this area are fundamental importance and relevance.

CONCLUSIONS

This systematic review was conducted based on articles that used animal models of ASD to facilitate the study and understanding of the neuromodulatory mechanisms involved in stereotypies. We consider that the articles included in this study have a low risk of bias and good internal validity, so it is very plausible that neurotransmitters play a central role in stereotypies in ASD. These types of studies and findings are critical in providing information that can be used in clinical applications, as well as providing new insights into the pathogenesis and management of ASD.

There is strong evidence of correlations between neurotransmitter levels in certain brain regions and the appearance of stereotypies in ASD. The most studied neurotransmitters that point to a significant impact in modulating these behaviors are serotonin, dopamine, GABA, glutamate, and acetylcholine. It is not yet possible to fully map the pathophysiological role of neurotransmitters and all their communication possibilities in each specific region of the brain.

The implication of the dopaminergic and serotoninergic pathways in ASD are to some extent well-proven, and drugs such as risperidone and Selective Serotonin Reuptake Inhibitors (SSRIs) have been used for some time to treat many of the symptoms present in autistic people. Concerning GABAergic neurotransmission, we may soon have available a drug that acts by potentiating this pathway, such as bumetanide.

The glutamatergic pathway contributes to the increase in stereotypes because an action that potentiates this pathway increases excitatory stimuli, but there is no antagonist drug of this pathway in use for the control of these movement disorders.

As for the cholinergic system, the deregulation of this system can result in important pathophysiological changes, including impacts on the dopaminergic system, which are directly linked to motor disorders. In individuals with ASD, the activation of the cholinergic system is related to the suppression of stereotyped behaviors. Preliminary studies point to the use of cholinesterase inhibitors in ASD, but with no evidence to reduce stereotypies.

REFERENCES

1.American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Association; 2013. https://doi.org/10.1176/appi.books.9780890425596

2.Péter Z, Oliphant ME, Fernandez TV. Motor Stereotypies: A Pathophysiological Review. Front Neurosci 2017;11:171. https://doi.org/10.3389/fnins.2017.00171

3.Ghanizadeh A. Clinical approach to motor stereotypies in autistic children. Iran J Ped 2010;20:149-59. https://pubmed.ncbi.nlm.nih.gov/23056697/

4.Muthugovindan D, Singer H. Motor stereotypy disorders. Curr Opin Neurol 2009;22:131-6. https://doi.org/10.1097/WCO.0b013e328326f6c8

5.Herbin M, Simonis C, Revéret L, Hackert R, Libourel P-A, Eugène D, *et al*. Dopamine Modulates Motor Control in a Specific Plane Related to Support. PLOS ONE 2016;11:e0155058. https://doi.org/10.1371/journal.pone.0155058

6.Schain RJ, Freedman DX. Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. J Ped 1961;58:315-20. https://doi.org/10.1016/s0022-3476(61)80261-8

7.Reiner A, Levitz J. Glutamatergic Signaling in the Central Nervous System: Ionotropic and Metabotropic Receptors in Concert. Neuron 2018;98:1080-98. https://doi.org/10.1016/j.neuron.2018.05.018

8.Nakanishi S. Molecular diversity of glutamate receptors and implications for brain function. Science 1992;258:597-603. https://doi.org/10.1126/science.1329206
9.Petroff OAC. Book Review: GABA and Glutamate in the Human Brain. Neurosci 2002;8:562-73. https://doi.org/10.1177/1073858402238515
10.Schür RR, Draisma LWR, Wijnen JP, Boks MP, Koevoets MGJC, Joëls M, *et al*. Brain GABA levels across psychiatric disorders: A systematic literature review and meta-analysis of1H-MRS studies. Hum Brain Mapp 2016;37:3337-52. https://doi.org/10.1002/hbm.23244
11.Lauder JM, Schambra UB. Morphogenetic roles of acetylcholine. Environ Health Perspec 1999;107(Suppl 1):65-9. https://doi.org/10.1289/ehp.99107s165
12.Karvat G, Kimchi T. Acetylcholine Elevation Relieves Cognitive Rigidity and Social Deficiency in a Mouse Model of Autism. Neuropsychopharmacol 2013;39:831-40. https://doi.org/10.1038/npp.2013.274
13.Haddad F, Sawalha M, Khawaja Y, Najjar A, Karaman R. Dopamine and Levodopa Prodrugs for the Treatment of Parkinson's Disease. Molecules 2017;23:40. https://doi.org/10.3390/molecules23010040
14.National Toxicology Program (NTP). Office of Health Assessment and Translation (OHAT): Risk of Bias Rating Tool for Human and Animal Studies. 2015. https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf
15.National Heart, Lung, and Blood Institute (NHLBI). Study quality assessment tools. Bethesda: U.S. Department of Health & Human Services. 2013. https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools
16.Amodeo DA, Rivera E, Cook EH, Sweeney JA, Ragozzino ME. 5HT2Areceptor blockade in dorsomedial striatum reduces repetitive behaviors in BTBR mice. Genes Brain Behav 2016;16:342-51. https://doi.org/10.1111/gbb.12343
17.Canal CE, Felsing DE, Liu Y, Zhu W, Wood JT, Perry CK, *et al*. An Orally Active Phenylaminotetralin-Chemotype Serotonin 5-HT_7 and 5-HT_{1A} Receptor Partial Agonist That Corrects Motor Stereotypy in Mouse Models. ACS Chem Neurosci 2015;6:1259-70. https://doi.org/10.1021/acschemneuro.5b00099
18.Mohammadi S, Asadi-Shekaari M, Basiri M, Parvan M, Shabani M, Nozari M. Improvement of autistic-like behaviors in adult rats prenatally exposed to valproic acid through early suppression of NMDA receptor function. Psychopharmacol 2019;237:199-208. https://doi.org/10.1007/s00213-019-05357-2
19.Jiang S, Xiao L, Sun Y, He M, Gao C, Zhu C, *et al*. The GABAB receptor agonist STX209 reverses the autism-like behaviour in an animal model of autism induced by prenatal exposure to valproic acid. Mol Med Rep 2022;25:5. https://doi.org/10.3892/mmr.2022.12670
20.Jiang S, He M, Xiao L, Sun Y, Ding J, Li W, *et al*. Prenatal GABAB Receptor Agonist Administration Corrects the Inheritance of Autism-Like Core Behaviors in Offspring of Mice Prenatally Exposed to Valproic

Acid. Front Psychiatr 2022;13:835993. https://doi.org/10.3389/fpsyt.2022.835993
21.Silverman JL, Pride MC, Hayes JE, Puhger KR, Butler-Struben HM, Baker S, *et al*. GABAB Receptor Agonist R-Baclofen Reverses Social Deficits and Reduces Repetitive Behavior in Two Mouse Models of Autism. Neuropsychopharmacol 2015;40:2228-39. https://doi.org/10.1038/npp.2015.66
22.Venkatachalam K, Eissa N, Awad MA, Jayaprakash P, Zhong S, Stölting F, *et al*. The histamine H3R and dopamine D2R/D3R antagonist ST-713 ameliorates autism-like behavioral features in BTBR T+tf/J mice by multiple actions. Biomed Pharmacother 2021;138:111517. https://doi.org/10.1016/j.biopha.2021.111517
23.Yang Y, Wang B, Zhong Z, Chen H, Ding W, Hoi MPM. Clonazepam attenuates neurobehavioral abnormalities in offspring exposed to maternal immune activation by enhancing GABAergic neurotransmission. Biochem Pharmacol 2021;192:114711. https://doi.org/10.1016/j.bcp.2021.114711
24.Yang J-Q, Yang C-H, Yin B-Q. Combined the GABA-A and GABA-B receptor agonists attenuates autistic behaviors in a prenatal valproic acid-induced mouse model of autism. Behav Brain Res 2021;403:113094. https://doi.org/10.1016/j.bbr.2020.113094
25.Yoshimura RF, Tran MB, Hogenkamp DJ, Ayala NL, Johnstone T, Dunnigan AJ, *et al*. Allosteric modulation of nicotinic and GABA A receptor subtypes differentially modify autism-like behaviors in the BTBR mouse model. Neuropharmacol 2017;126:38-47. https://doi.org/10.1016/j.neuropharm.2017.08.029
26.Folk GE, Long JP. Serotonin as a neurotransmitter: A review. Comp Biochem Physiol C Comp Pharmacol Toxicol 1988;91:251-7. https://doi.org/10.1016/0742-8413(88)90193-4
27.Lovenberg W, Jequier E, Sjoerdsma A. Tryptophan Hydroxylation: Measurement in Pineal Gland, Brainstem, and Carcinoid Tumor. Science 1967;155:217-9. https://doi.org/10.1126/science.155.3759.217
28.Amodeo DA, Rivera E, Dunn JT, Ragozzino ME. M100907 attenuates elevated grooming behavior in the BTBR mouse. Behav Brain Res 2016;313:67-70. https://doi.org/10.1016/j.bbr.2016.06.064
29.Amodeo DA, Oliver B, Pahua A, Hitchcock K, Bykowski A, Tice D, *et al*. Serotonin 6 receptor blockade reduces repetitive behavior in the BTBR mouse model of autism spectrum disorder. Pharmacol Biochem Behav 2021;200:173076. https://doi.org/10.1016/j.pbb.2020.173076
30.Silverman JL, Tolu SS, Barkan CL, Crawley JN. Repetitive Self-Grooming Behavior in the BTBR Mouse Model of Autism is Blocked by the mGluR5 Antagonist MPEP. Neuropsychopharmacol 2009;35:976–89. https://doi.org/10.1038/npp.2009.201
31.Raote I, Bhattacharya A, Panicker MM, Chattopadhyay A (eds). Serotonin 2A (5-HT2A) Receptor Function: Ligand-Dependent Mechanisms and Pathways. In: Serotonin Receptors in Neurobiology. Boca Raton: CRC Press/Taylor & Francis; 2007; chap 6. https://pubmed.ncbi.nlm.nih.gov/21204452/

32.Delmonte S, Gallagher L, O'Hanlon E, McGrath J, Balsters JH. Functional and structural connectivity of frontostriatal circuitry in Autism Spectrum Disorder. Front Hum Neurosci 2013;7:430. https://doi.org/10.3389/fnhum.2013.00430
33.Woolley ML, Marsden CA, Fone KCF. 5-ht6 receptors. Current drug targets CNS Neurolog Disord 2004;3:59-79. https://doi.org/10.2174/1568007043482561
34.Pompeiano M, Palacios J, Mengod G. Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: correlation with receptor binding. J Neurosci 1992;12:440-53. https://doi.org/10.1523/JNEUROSCI.12-02-00440.1992
35.Spooren W, Lindemann L, Ghosh A, Santarelli L. Synapse dysfunction in autism: a molecular medicine approach to drug discovery in neurodevelopmental disorders. Trend Pharmacol Sci 2012;33:669-84. https://doi.org/10.1016/j.tips.2012.09.004
36.Thomas D, Hagan J. 5-HT7 Receptors. Curr Drug Target CNS Neurolog Disord 2004;3:81-90. https://doi.org/10.2174/1568007043482633
37.Guillery RW, Sherman SM. The thalamus as a monitor of motor outputs. Philos Trans R Soc Lond B Biol Sci 2002;357:1809-21. https://doi.org/10.1098/rstb.2002.1171
38.Gu F, Chauhan V, Chauhan A. Monoamine oxidase-A and B activities in the cerebellum and frontal cortex of children and young adults with autism. J Neurosci Res 2017;95:1965-72. https://doi.org/10.1002/jnr.24027
39.Juárez Olguín H, Calderón Guzmán D, Hernández García E, Barragán Mejía G. The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. Oxid Med Cel Longev 2016;2016:1-13. https://doi.org/10.1155/2016/9730467
40.Howes O, McCutcheon R, Stone J. Glutamate and dopamine in schizophrenia: An update for the 21st century. J Psychopharmacol 2015;29:97-115. https://doi.org/10.1177/0269881114563634
41.Tibbo P, Warneke L. Obsessive-compulsive disorder in schizophrenia: epidemiologic and biologic overlap. J Psychiatr Neurosci 1999;24:15-24. https://pubmed.ncbi.nlm.nih.gov/9987204/
42.De Corte BJ, Wagner LM, Matell MS, Narayanan NS. Striatal dopamine and the temporal control of behavior. Behav Brain Res 2019;356:375-9. https://doi.org/10.1016/j.bbr.2018.08.030
43.Lee Y, Kim H, Han P-L. Striatal Inhibition of MeCP2 or TSC1 Produces Sociability Deficits and Repetitive Behaviors. Exp Neurobiol 2018;27:539-49. https://doi.org/10.5607/en.2018.27.6.539
44.Wang W, Li C, Chen Q, van der Goes M-S, Hawrot J, Yao AY, *et al*. Striatopallidal dysfunction underlies repetitive behavior in Shank3-deficient model of autism. J Clin Investig 2017;127:1978-90. https://doi.org/10.1172/JCI87997
45.Staal WG. Autism, DRD3 and repetitive and stereotyped behavior, an overview of the current knowledge. Eur Neuropsychopharmacol 2015;25:1421-6. https://doi.org/10.1016/j.euroneuro.2014.08.011

46.Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine Receptors: from Structure to Function. Physiol Rev 1998;78:189-225. https://doi.org/10.1152/physrev.1998.78.1.189
47.Wang S, Che T, Levit A, Shoichet BK, Wacker D, Roth BL. Structure of the D2 dopamine receptor bound to the atypical antipsychotic drug risperidone. Nature 2018;555:269-73. https://doi.org/10.1038/nature25758
48.Sander K, Kottke T, Stark H. Histamine H3 Receptor Antagonists Go to Clinics. Bio Pharmac Bull 2008;31:2163-81. https://doi.org/10.1248/bpb.31.2163
49.Shan L, Bao A-M, Swaab DF. The human histaminergic system in neuropsychiatric disorders. Trend Neurosci 2015;38:167-77. https://doi.org/10.1016/j.tins.2014.12.008
50.Hamilton PJ, Campbell NG, Sharma S, Erreger K, Herborg Hansen F, Saunders C, *et al*. De novo mutation in the dopamine transporter gene associates dopamine dysfunction with autism spectrum disorder. Mol Psychiatr 2013;18:1315-23. https://doi.org/10.1038/mp.2013.102
51.DiCarlo GE, Aguilar JI, Matthies HJ, Harrison FE, Bundschuh KE, West A, *et al*. Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. J Clin Investig 2019;129:3407-19. https://doi.org/10.1172/JCI127411
52.Enna SJ. The GABA Receptors. 3rd ed. GABA Recep 2007; pp1-21. https://doi.org/10.1007/978-1-59745-465-0_1
53.Pizzarelli R, Cherubini E. Alterations of GABAergic Signaling in Autism Spectrum Disorders. Neural Plast 2011;2011:1-12. https://doi.org/10.1155/2011/297153
54.Chao H-T, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, *et al*. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 2010;468:263-9. https://doi.org/10.1038/nature09582
55.Cogram P, Deacon RMJ, Warner-Schmidt JL, von Schimmelmann MJ, Abrahams BS, During MJ. Gaboxadol Normalizes Behavioral Abnormalities in a Mouse Model of Fragile X Syndrome. Front Behav Neurosci 2019;13:141. https://doi.org/10.3389/fnbeh.2019.00141
56.Ebihara K, Fujiwara H, Awale S, Dibwe DF, Araki R, Yabe T, *et al*. Decrease in endogenous brain allopregnanolone induces autism spectrum disorder (ASD)-like behavior in mice: A novel animal model of ASD. Behav Brain Res 2017;334:6-15. https://doi.org/10.1016/j.bbr.2017.07.019
57.Kotajima-Murakami H, Hagihara H, Sato A, Hagino Y, Tanaka M, Katoh Y, *et al*. Exposure to GABAA Receptor Antagonist Picrotoxin in Pregnant Mice Causes Autism-Like Behaviors and Aberrant Gene Expression in Offspring. Front Psychiatr 2022;13:821354. https://doi.org/10.3389/fpsyt.2022.821354
58.Zhang L, Huang C-C, Dai Y, Luo Q, Ji Y, Wang K, *et al*. Symptom improvement in children with autism spectrum disorder following bumetanide administration is associated with decreased

GABA/glutamate ratios. Translat Psychiatr 2020;10:1-12. https://doi.org/10.1038/s41398-020-0692-2
59.Sohal VS, Rubenstein JLR. Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. Mol Psychiatr 2019;24:1248-57. https://doi.org/10.1038/s41380-019-0426-0
60.Rubenstein JLR, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003;2:255-67. https://doi.org/10.1034/j.1601-183x.2003.00037.x
61.Sears SM, Hewett SJ. Influence of glutamate and GABA transport on brain excitatory/inhibitory balance. Exp Bioa Med 2021;246:1069-83. https://doi.org/10.1177/1535370221989263
62.Nelson SB, Valakh V. Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders. Neuron 2015;87:684-98. https://doi.org/10.1016/j.neuron.2015.07.033
63.Sadybekov A, Tian C, Arnesano C, Katritch V, Herring BE. An autism spectrum disorder-related de novo mutation hotspot discovered in the GEF1 domain of Trio. Nature Comm 2017;8:1. https://doi.org/10.1038/s41467-017-00472-0
64.Schmidt S, Debant A. Function and regulation of the Rho guanine nucleotide exchange factor Trio. Small GTPases 2014;5:e983880. https://doi.org/10.4161/sgtp.29769
65.Horder J, Petrinovic MM, Mendez MA, Bruns A, Takumi T, Spooren W, *et al*. Glutamate and GABA in autism spectrum disorder—a translational magnetic resonance spectroscopy study in man and rodent models. Translat Psychiatr 2018;8:106. https://doi.org/10.1038/s41398-018-0155-1
66.Kim H, Kim T-K, Kim J-E, Park J-Y, Lee Y, Kang M, *et al*. Adenylyl cyclase-5 in the dorsal striatum function as a molecular switch for the generation of behavioral preferences for cue-directed food choices. Mol Brain 2014;7:77. https://doi.org/10.1186/s13041-014-0077-7
67.Kim H, Lee Y, Park J-Y, Kim J-E, Kim T-K, Choi J, *et al*. Loss of Adenylyl Cyclase Type-5 in the Dorsal Striatum Produces Autistic-Like Behaviors. Mol Neurobiol 2017;54:7994-8008. https://doi.org/10.1007/s12035-016-0256-x
68.Presti MF, Mikes HM, Lewis MH. Selective blockade of spontaneous motor stereotypy via intrastriatal pharmacological manipulation. Pharmacol Biochem Behav 2003;74:833-9. https://doi.org/10.1016/s0091-3057(02)01081-x
69.Burket JA, Herndon AL, Winebarger EE, Jacome LF, Deutsch SI. Complex effects of mGluR5 antagonism on sociability and stereotypic behaviors in mice: Possible implications for the pharmacotherapy of autism spectrum disorders. Brain Res Bull 2011;86:152-8. https://doi.org/10.1016/j.brainresbull.2011.08.001
70.Mehta MV, Gandal MJ, Siegel SJ. mGluR5-Antagonist Mediated Reversal of Elevated Stereotyped, Repetitive Behaviors in the VPA Model of Autism. PLoS ONE 2011;6:e26077. https://doi.org/10.1371/journal.pone.0026077

71.Aguilar-Valles A, Matta-Camacho E, Khoutorsky A, Gkogkas C, Nader K, Lacaille J-C, *et al*. Inhibition of Group I Metabotropic Glutamate Receptors Reverses Autistic-Like Phenotypes Caused by Deficiency of the Translation Repressor eIF4E Binding Protein 2. J Neurosci 2015;35:11125-32. https://doi.org/10.1523/JNEUROSCI.4615-14.2015
72.Kang J, Kim E. Suppression of NMDA receptor function in mice prenatally exposed to valproic acid improves social deficits and repetitive behaviors. Front Mol Neurosci 2015;8:17. https://doi.org/10.3389/fnmol.2015.00017
73.Wu H-F, Chen PS, Hsu Y-T, Lee C-W, Wang T-F, Chen Y-J, *et al*. D-Cycloserine Ameliorates Autism-Like Deficits by Removing GluA2-Containing AMPA Receptors in a Valproic Acid-Induced Rat Model. Mol Neurobio 2017;55:4811-24. https://doi.org/10.1007/s12035-017-0685-1
74.Adil KJ, Gonzales EL, Remonde CG, Boo K-J, Jeon SJ, Shin CY. Autism-Like Behavioral Phenotypes in Mice Treated with Systemic N-Methyl-D-Aspartate. Biomol Therap 2022;30:232-7. https://doi.org/10.4062/biomolther.2021.133
75.Kim S, Kim DG, Gonzales E luck, Mabunga DFN, Shin D, Jeon SJ, *et al*. Effects of Intraperitoneal N-methyl-D-aspartate (NMDA) Administration on Nociceptive/Repetitive Behaviors in Juvenile Mice. Biomol Therap 2019;27:168-77. https://doi.org/10.4062/biomolther.2018.230
76.Schade S, Paulus W. D-Cycloserine in Neuropsychiatric Diseases: A Systematic Review. Inter J Neuropsychopharmacol 2015;19:pyv102. https://doi.org/10.1093/ijnp/pyv102
77.Mukaetova-Ladinska EB, Westwood J, Perry EK. Cholinergic Component of Autism Spectrum Disorder. Neurochem Basis Autism 2010;1:129-61. https://doi.org/10.1007/978-1-4419-1272-5_9
78.Brown DA. Muscarinic Acetylcholine Receptors (mAChRs) in the Nervous System: Some Functions and Mechanisms. J Mol Neurosci 2010;41:340-6. https://doi.org/10.1007/s12031-010-9377-2
79.Lester DB, Rogers TD, Blaha CD. Acetylcholine-Dopamine Interactions in the Pathophysiology and Treatment of CNS Disorders. CNS Neurosci Therap 2010;16:137-62. https://doi.org/10.1111/j.1755-5949.2010.00142.x
80.Amodeo DA, Yi J, Sweeney JA, Ragozzino ME. Oxotremorine treatment reduces repetitive behaviors in BTBR T+ tf/J mice. Front Synaptic Neurosci 2014;6:17. https://doi.org/10.3389/fnsyn.2014.00017
81.Kim JW, Seung H, Kwon KJ, Ko MJ, Lee EJ, Oh HA, *et al*. Subchronic treatment of donepezil rescues impaired social, hyperactive, and stereotypic behavior in valproic acid-induced animal model of autism. PLoS One 2014;9:e104927. https://doi.org/10.1371/journal.pone.0104927