University of Pécs

Faculty of Medicine

Ph.D. thesis

THE EFFECT OF THE INTRAAMYGDALOID OXYTOCIN FOR THE BEHAVIOR IN VALPROATE INDUCED AUTISM RAT MODEL

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1. Introduction

Neurodevelopmental disorders occur across cultures and populations worldwide; however, reported prevalence rates are influenced by diagnostic practices, age, and socioeconomic factors. These conditions are consistently more common in males. Their global prevalence in the population under 18 years of age ranges between 4.7–18,3%, likely reflecting methodological differences. Comorbid neurodevelopmental disorders are common [1]. According to a U.S. study, among individuals aged 3–17 years, the prevalence of attention-deficit/hyperactivity disorder was 8.5%, autism spectrum disorder 2.9%, intellectual disability 1.4%, and learning disorders 6.4%. Prevalence was lower among children of married parents and those living with siblings, whereas higher rates were observed in lower socioeconomic strata [2]. Between 1990 and 2021, the prevalence of autism spectrum disorder increased, underscoring the need for a more precise understanding of pathomechanisms and the identification of novel therapeutic targets [3].

2. Autism Spectrum Disorder

Autism spectrum disorder is a pervasive neurodevelopmental condition characterized by impairments in social communication and interaction, along with restricted and repetitive patterns of behavior [4]. Its worldwide prevalence is approximately 1% [5]; in Hungary it is around 1.4%, likely underestimated due to limitations of the healthcare system [6]. Both genetic and environmental factors contribute to its development [7]. Variations and epigenetic modifications of the oxytocin receptor gene may play a role in its pathogenesis. Hypermethylation alone cannot be regarded as a definitive risk factor: in adults it correlates with a greater number of quantitative symptoms, whereas in children hypomethylation appears to confer increased risk [8]. Among environmental influences, intrauterine valproate exposure is particularly notable, as several studies indicate an elevated risk of developing the disorder [9,10]. Oxytocin has emerged as a promising neuropeptide for alleviating core symptoms [11]. Controlled clinical trials have shown that intravenous administration improved symptoms, particularly social behavior [12]. We previously demonstrated that microinjection of 10 ng oxytocin into the central amygdala exerts a positive reinforcing effect and enhances memory performance in neurotypical rats [13,14]. The dopaminergic system has also been implicated in this reinforcing effect [15]. A review analyzing 132 publications reported that administration of 400–600 mg/kg valproate on gestational day 12.5 induces autism-like behavioral alterations in offspring, with abnormalities detectable across multiple behavioral domains, supporting the model's validity [16].

Intrauterine valproate exposure resulted in reduced ultrasonic vocalization on postnatal day 5, with both call number and duration decreased compared to neurotypical controls. Valproate-exposed animals exhibited reduced dendritic branching and shorter neurites in primary cortical

and parvalbumin-positive neurons, whereas GABAergic neurons showed increased arborization and somatostatin-positive neurons remained unchanged. These findings suggest that prenatal valproate exposure alters cortical neuronal morphology and GABAergic signaling markers, potentially contributing to early vocalization and motor abnormalities [17]. The model also demonstrates gut microbiome dysbiosis in addition to behavioral and neuroanatomical changes [18]. In a previous study, probiotic treatment attenuated social behavioral deficits, supporting an interaction between the microbiome and the mature brain [19]. The amygdala was selected as the target region due to its central role in social behavior, and its dysfunction may contribute to the emergence of ASD-related phenotypes [20].

3. Objectives

The aim of our study was to determine how intraamygdaloid oxytocin influences reinforcement processes, anxiety, and social behavior, and to clarify the receptor mechanisms mediating these effects in a valproate-induced autism model.

3.1 Reinforcement

To determine whether oxytocin administered into the central amygdala exerts positive or negative reinforcing effects in prenatally valproate-treated male Wistar rats, and whether these effects are oxytocin receptor dependent and modifiable through dopamine D2 receptor mechanisms.

3.2 Anxiety

To assess the effect of intraamygdaloid oxytocin on anxiety-like behavior in animals exhibiting autistic features and to elucidate the receptor mechanisms involved.

3.3 Social behavior

To determine the role of intraamygdaloid oxytocin in regulating social behavior in control and valproate-treated animals and to examine the receptor mechanisms underlying these effects.

4. Materials and Methods

4.1 Experimental animals

A total of 186 male Wistar rats were used (social interaction: 65; elevated plus maze: 42; conditioned place preference: 79). Animals were either neurotypical or displayed autistic-like features. Experiments were conducted in accordance with institutional, national, and international animal welfare regulations and the ARRIVE guidelines. Ethical approval numbers were BA02/2000-8/2012, BA02/2000-64/2017 and BA02/2000-04/2021 (University of Pécs, approved by the Scientific Ethical Council for Animal Experiments). The autistic-like phenotype was induced by intraperitoneal administration of 500 mg/kg valproate on gestational day 12.5 [21]. Early neurodevelopment was assessed using the righting reflex, negative geotaxis, and ultrasonic vocalization following maternal separation. Reduced ultrasonic vocalization is considered an indicator of socio-communicative impairment [22]. At four weeks of age, social interaction and open field tests were performed. Inclusion criteria were reduced social activity and increased repetitive behavior relative to controls.

4.2 Stereotaxic surgery

At a body weight of 270–290 g, bilateral guide cannulae were implanted above the central amygdala according to the Paxinos–Watson atlas [23]. Surgery was performed under ketamine and diazepam anesthesia with antibiotic prophylaxis. Behavioral testing was conducted under standardized conditions after at least six days of recovery.

4.3 Drug administration

Five minutes before testing, bilateral microinjections were administered into the central amygdala (0.4 μL per side): 10 ng oxytocin, 20 ng oxytocin receptor antagonist, 4 μg dopamine D2 receptor antagonist, or vehicle. Injections were delivered at a constant rate, and cannulae were left in place for an additional 60 seconds to ensure diffusion. Groups were formed while controlling for litter effects [24]. Behavior was analyzed from video recordings.

4.4 Histology

At the end of experiments, animals were deeply anesthetized and transcardially perfused. Coronal sections (40 μm) were stained with Cresyl violet, and injection sites were reconstructed

using a brain atlas [23]. Only data from animals with correct cannula placement were included in the analysis.

4.5 Statistical analysis

Data normality was assessed prior to analysis. One- and two-way analyses of variance followed by Tukey's post hoc tests were applied. Results are presented as mean ± standard error; significance level was set at $p < 0.05$.

5. Behavioral Tests

5.1 Conditioned place preference

In the oxytocin and oxytocin receptor antagonist experiment, two-way analysis of variance revealed significant main effects of trial [$F(1,34) = 5.746$, $p < 0.05$] and treatment [$F(3,34) = 4.883$, $p < 0.05$], as well as a significant interaction [$F(3,32) = 6.189$, $p < 0.01$]. In the valproate + 10 ng oxytocin group ($n = 7$), time spent in the treatment quadrant on the test day was significantly increased compared to the control group ($n = 6$, $p < 0.05$) and habituation values ($p < 0.05$), indicating a positive reinforcing effect. Oxytocin receptor antagonist pretreatment ($n = 7$) abolished this effect ($p < 0.05$), whereas antagonist alone ($n = 7$) did not alter place preference compared to controls ($n = 6$, N.S.), but differed significantly from the oxytocin-treated group ($p < 0.05$).

In the dopamine D2 receptor antagonist experiment, analysis of variance revealed significant effects of trial [$F(1,35) = 4.648$, $p < 0.05$], treatment [$F(3,35) = 3.441$, $p < 0.05$], and interaction [$F(3,35) = 6.536$, $p < 0.01$]. In the valproate + 10 ng oxytocin group ($n = 7$), test phase preference exceeded that of controls ($n = 8$, $p < 0.05$) and habituation values ($p < 0.05$). Dopamine D2 receptor antagonist pretreatment ($n = 7$) eliminated the positive reinforcing effect of oxytocin ($p < 0.05$). Antagonist alone ($n = 7$) did not alter place preference compared to controls ($n = 8$), but differed significantly from the oxytocin-treated group ($p < 0.05$).

5.2 Elevated plus maze

Anxiety-like behavior was assessed using the elevated plus maze. The apparatus consisted of a grey-painted wooden structure with four arms: two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm), elevated one meter above the floor. Following drug administration, animals were placed on the central platform facing a closed arm. During the five-minute test, the number of arm entries, the time spent in the open and closed arms, and the frequency of head-dipping were recorded. Each animal participated in the test only once [25]. From the forty-

two animals, five groups were formed: control, valproate, valproate + 10 ng oxytocin, valproate + oxytocin receptor antagonist + oxytocin, and valproate + oxytocin receptor antagonist.

For time spent in the open arms, one-way analysis of variance revealed a significant difference [$F(4,33) = 4.387$, $\eta^2 = 0.347$, $p < 0.01$]. The valproate + 10 ng oxytocin group ($n = 8$) spent significantly more time in the open arms than the valproate group ($p < 0.05$), the valproate + oxytocin receptor antagonist + oxytocin group ($p < 0.05$), and the valproate + oxytocin receptor antagonist group ($p < 0.05$). No difference was observed between the control and the valproate + oxytocin groups. Pretreatment with the oxytocin receptor antagonist prevented the anxiolytic effect of oxytocin, while the antagonist alone did not modify behavior. Control animals spent more time in the open arms than the valproate-treated group ($p < 0.05$).

A similar pattern was observed for the number of open-arm entries [$F(4,33) = 3.162$, $\eta^2 = 0.265$, $p < 0.05$]. The valproate + oxytocin group entered the open arms more frequently than the other valproate-treated groups ($p < 0.05$), whereas no difference was found between the control and the valproate + oxytocin groups.

The number of head-dipping events also differed significantly between groups [$F(4,33) = 12.345$, $\eta^2 = 0.599$, $p < 0.001$]. The valproate + oxytocin group showed a higher frequency of head-dipping than the other valproate-treated groups ($p < 0.05$). Pretreatment with the oxytocin receptor antagonist abolished this effect. Control animals also displayed more head-dipping behavior than the valproate group ($p < 0.05$).

5.3 Social interaction test

Social interaction was examined using a three-chamber apparatus (150 × 40 × 40 cm) [26]. One side chamber contained a stimulus animal, while the other contained an empty cage. Sociability was determined as the ratio of time spent in the social and non-social zones [27]. From sixty-five animals, eight groups were formed: neurotypical control, neurotypical + oxytocin, neurotypical + oxytocin receptor antagonist + oxytocin, neurotypical + oxytocin receptor antagonist, valproate, valproate + oxytocin, valproate + oxytocin receptor antagonist + oxytocin, and valproate + oxytocin receptor antagonist.

In neurotypical animals, one-way analysis of variance revealed a significant difference [$F(3,29) = 6.402$, $p < 0.01$]. The 10 ng oxytocin group spent significantly more time in the social zone than the control or oxytocin receptor antagonist–treated groups ($p < 0.05$).

In valproate-treated animals, two-way analysis of variance showed a significant main effect of intrauterine treatment [$F(1,36) = 39.895$, $p < 0.05$], a significant effect of intraamygdaloid treatment [$F(3,36) = 15.575$, $p < 0.05$], and a significant interaction [$F(3,35) = 20.888$, $p < 0.05$]. The valproate + oxytocin group spent more time in the social zone than the other valproate-treated groups ($p < 0.05$), and did not differ from the neurotypical control group. Pretreatment

with the oxytocin receptor antagonist prevented the effect of oxytocin, while the antagonist alone did not modify social behavior.

The time spent in the non-social zone and the sociability index showed a consistent pattern: oxytocin increased social preference, this effect was prevented by the oxytocin receptor antagonist, while the antagonist alone did not influence behavior.

6. Discussion

6.1 Conditioned place preference

Based on our previous findings, intraamygdaloid administration of 10 ng oxytocin induces a positive reinforcing effect in the conditioned place preference test in neurotypical rats, whereas a higher dose (100 ng) does not influence place preference [14]. This reinforcing effect of oxytocin can be prevented by dopamine D2 receptor antagonist pretreatment [28], indicating the involvement of the dopaminergic system. In the present study we observed that the rewarding effect of intraamygdaloid oxytocin is also preserved in the valproate-induced rat model of autism spectrum disorder [28]. This is particularly notable in light of the hypothesis that the reward system exhibits hypoactivity in autism spectrum disorder [29]. Our results demonstrate that microinjection of 10 ng oxytocin into the central amygdala exerts a reinforcing effect.

The positive reinforcing action of oxytocin has been described in several brain regions [14,30], and peripheral administration has also been shown to enhance motivation in the conditioned place preference paradigm [31]. The development of place preference requires both motivational and memory processes; during the test the animal must recall the treatment-associated quadrant, therefore the paradigm also reflects memory-dependent conditioning [32]. The dopaminergic system is known to play a key role in reinforcement, learning, and memory processes [33], and its dysfunction has been proposed as one of the mechanisms underlying autism spectrum disorder [34].

Our hypothesis is that the positive reinforcing effect of oxytocin in rats displaying autistic-like traits is mediated at least partly through the mesolimbic dopaminergic system. Dysfunction of the mesolimbic pathway may contribute to impairments in social skills, whereas alterations in the nigrostriatal pathway may lead to stereotyped behavior [34]. Because the mesolimbic system plays a central role in motivation and reward processing, its disruption may result in reduced reward valuation and altered motivation. This is consistent with the social motivation theory, which proposes that in autism spectrum disorder the rewarding value of social stimuli is diminished, leading to impairments in social cognition and social functioning [34]. Reduced dopamine release and hypoactivity of the reward system have been described, affecting the processing of both social and non-social rewards [29].

The oxytocinergic and dopaminergic systems are closely interconnected functionally [35]. Co-localization of dopamine D2 and oxytocin receptors has been demonstrated in the central

amygdala and the striatum [36]. Dopaminergic neurons of the ventral tegmental area express oxytocin receptors and project to limbic structures [37]. Oxytocin can modulate dopamine release within the mesolimbic system [37]. Oxytocin–dopamine D2 receptor heterocomplexes have been described in the central amygdala, and activation of dopamine D2 receptors may enhance the effects of oxytocin [38]. Activation of the oxytocin receptor promoter may further increase dopamine D2 receptor signaling through allosteric facilitation [38]. Based on these mechanisms, the blockade of the reinforcing effect by dopamine D2 receptor antagonist pretreatment may reflect inhibition of oxytocin–dopamine D2 heterocomplex function. Nevertheless, further studies are required to clarify the precise role of the mesolimbic system in this process.

6.2 Elevated plus maze

The results of the elevated plus maze confirm that prenatal valproate treatment increases anxiety-like behavior [21], which is accompanied by enhanced amygdala reactivity [39]. Oxytocin has been shown to modulate anxiety within the amygdala [15]. We previously demonstrated that 10 ng oxytocin exerts an anxiolytic effect in neurotypical male rats [40], and in the present study a similar effect was observed in valproate-treated animals. Intraamygdaloid oxytocin increased both the time spent in the open arms and the number of open-arm entries [41], and also increased the frequency of head-dipping, a behavioral indicator of reduced anxiety [25]. This effect proved to be oxytocin receptor specific, as pretreatment with an oxytocin receptor antagonist abolished it [41].

Unlike peptide antagonists, the use of a selective non-peptide antagonist allowed the assessment of receptor specificity, since peptide antagonists may also exhibit partial agonist properties [42]. These findings suggest that oxytocin may reduce anxiety through modulation of amygdala activity [43].

6.3 Social interaction

In the social interaction test, reduced social behavior was observed in the valproate model [44]. Intraamygdaloid oxytocin treatment increased the duration of social interaction without affecting the distance traveled, indicating a specific social effect [44]. Administration of the oxytocin receptor antagonist alone did not alter the time spent in social interaction; however, when given as a pretreatment it prevented the effect of oxytocin, confirming a receptor-mediated mechanism [44]. If the antagonist had failed to block the effect, a potential role of vasopressin receptors could have been considered, as the two systems show structural and functional overlap [45].

The valproate model is widely accepted for preclinical investigation of autism spectrum disorder [46]. In this model, decreased oxytocin mRNA levels, fewer oxytocin-immunoreactive

cells, and lower cerebrospinal fluid oxytocin concentrations have been reported [46]. Alterations of the oxytocin system have also been demonstrated in both monogenic and polygenic models of autism spectrum disorder [47]. Clinical data suggest that intranasal oxytocin may improve social functioning in some cases, although the long-term effects and optimal dosing require further investigation [48]. Anxiety is a frequent comorbidity of autism spectrum disorder [49], significantly impairing quality of life [50] and being associated with poorer social and academic performance [51]. Oxytocin has been implicated both in social reinforcement learning [52] and in the modulation of empathy and trust [53].

7. Conclusion

7.1 Administration of 10 ng oxytocin into the central amygdala induced a positive reinforcing effect in prenatally valproate-treated male Wistar rats exhibiting autistic-like traits. The effect was oxytocin receptor specific and abolished by dopamine D2 receptor antagonist pretreatment, indicating involvement of the dopaminergic system.

7.2 Intraamygdaloid oxytocin reduced anxiety-like behavior in the valproate-induced autism model. It increased open-arm time, open-arm entries, and head-dipping frequency in the elevated plus maze in an oxytocin receptor–dependent manner.

7.3 Microinjection of 10 ng oxytocin into the central nucleus of the amygdala increased time spent in social interaction in the model, likely via oxytocin receptor–mediated mechanisms.

Overall, intraamygdaloid oxytocin favorably modulated specific behavioral dimensions of autism spectrum disorder in the valproate-induced animal model. Further preclinical and clinical investigations are required to elucidate the precise mechanisms involved.

8. References

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9. Publications

The thesis is based on these publications:

1. László, K.; Vörös, D.; Kiss, O.; László, B.R.; Ollmann, T.; Péczely, L.; Mintál, K.; Tóth, A.; Kovács, A.; Zagoracz, O.; et al. The Role of Intraamygdaloid Oxytocin and D2 Dopamine Receptors in Reinforcement in the Valproate-Induced Autism Rat Model. Biomedicines 2022, 10, Paper:, 2309.

Q1; IF: 4,7

2. László, K.; Kiss, O.; Vörös, D.; Mintál, K.; Ollmann, T.; Péczely, L.; Kovács, A.; Zagoracz, O.; Kertes, E.; Kállai, V.; et al. Intraamygdaloid Oxytocin Reduces Anxiety in the Valproate-Induced Autism Rat Model. Biomedicines 2022, 10, Paper: 405.

Q1; IF:4,7

3. Vörös, D.; Kiss, O.; Ollmann, T.; Mintál, K.; Péczely, L.; Zagoracz, O.; Kertes, E.; Kállai, V.; László, B.R.; Berta, B.; et al. Intraamygdaloid Oxytocin Increases Time Spent on Social Interaction in Valproate-Induced Autism Animal Model. Biomedicines 2023, 11, 1802.

Q1; IF: 3,9

Further publications:

1. Vörös, Dávid ; Kiss, Orsolya ; Taigiszer, Márton ; László, Bettina Réka ; Ollmann, Tamás ; Péczely, László ; Zagorácz, Olga ; Kertes, Erika ; Kállai, Veronika ; Berta, Beáta et al. The role of intraamygdaloid oxytocin in spatial learning and avoidance learning PEPTIDES 175 Paper: 171169 , 8 p. (2024) Q2; IF: 2,9

2. Mihalj, Denisa ; Laszlo, Kristof ; Havranek, Tomas ; Voros, David ; Kupkova, Kristina ; Bacova, Zuzana ; Bakos, Jan: Prenatal Valproate Exposure Affects Cortical Neurite Branching, GABAergic Markers, Motor Reflexes and Ultrasonic Vocalizations in the Male Rat Pups JOURNAL OF NEUROCHEMISTRY 169 : 8 Paper: e70184 , 11 p. (2025) Q1; IF: 4,0

3. Zagorácz, Olga ; Ollmann, Tamás ; Péczely, László ; László, Kristóf ; Kovács, Anita ; Berta, Beáta ; Kállai, Veronika ; Kertes, Erika ; Vörös, Dávid ; Dusa, Daniella et al. A single injection of neuropeptide QRFP in the lateral hypothalamus decreased food intake. JOURNAL OF PSYCHOPHARMACOLOGY 39 : 3 pp. 254-264. , 11 p. (2025) Q1; IF: 5,5

4. László, Kristóf ; Vörös, Dávid ; Correia, Pedro ; Fazekas, Csilla Lea ; Török, Bibiána ; Plangár, Imola ; Zelena, Dóra Vasopressin as Possible Treatment Option in Autism Spectrum Disorder

BIOMEDICINES 11 : 10 Paper: 2603 , 37 p. (2023) Q1; IF 3,9

5. Berta, B ; Kertes, E ; Zagoracz, O ; Péczely, L ; Ollmann, T ; László, K ; Kállai, V ; Vörös, D ; Szabó, Á ; Lénárd, L :Effects of the dopamine D1-like antagonist SCH 23390 microinjected

into the prefrontal cortex on hedonic evaluation of tastant. In: 51st Meeting of the European Brain and Behaviour Society (2025) Paper: P3.17

6. Mintál, K ; Hormay, E ; László, K ; Kocsis, B ; Vörös, D ; Győrfi, N ; Vizvári, Z ; Cserjesi, R ; Lénárd, L ; Karádi, Z et al. Investigation of the effect of probiotic administration in a valproic acid-induced autism rat model. In: Fourth Symposium on Super-resolution and Advanced Fluorescence Microscopy and István Ábrahám Memorial Workshop (2025) 72 p. pp. 56-56. , 1 p.

7. László, K ; Vörös, D ; Kiss, O ; László, B ; Ollmann, T ; Péczely, L ; Mintál, K ; Tóth, A ; Kovács, A ; Zagoracz, O et al. The intraamydaloid oxytocin ameliorates some autistic-like symptoms in valproate-induced autism rodent model. In: International Neuroscience Conference, Pécs 2024 : Abstract book Pécs, Magyarország (2024) 302 p. p. 20 Paper: S2.04

8. Vörös, D ; Kiss, O ; Ollmann, T ; Mintál, K ; Péczely, L ; Zagoracz, O ; Kertes, E ; Kállai, V ; László, B ; Berta, B et al. Effect of intraamygdaloid oxytocin on social interaction in valproate-induced autism model. In: International Neuroscience Conference, Pécs 2024 : Abstract book Pécs, Magyarország (2024) 302 p. p. 237 Paper: P8.09

9. Zagorácz, O ; Ollmann, T ; Péczely, L ; László, K ; Kovács, A ; Berta, B ; Kállai, V ; Kertes, E ; Vörös, D ; Dusa, D et al. Neuropeptide QRFP enhances memory in passive avoidance paradigm. In: International Neuroscience Conference, Pécs 2024 : Abstract book Pécs, Magyarország (2024) 302 p. p. 195 Paper: P6.08

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11. László, K ; Kiss, O ; Vörös, D ; Mintál, K ; Ollmann, T ; Péczely, L ; Kovács, A ; Zagoracz, O ; Kertes, E ; Kállai, V et al. Behavioural effects of intraamygdaloid oxytocin in valproate induced autism rat model. In: International Neuroscience Meeting, Budapest 2022 : IBRO Workshop : 27-28 January 2022 Budapest Hungary : Abstract book (2022) 277 p. Paper: P7.03

12. Vörös, D.; Kiss, O.; Ollman, T.; Péczely, L.; Zagorácz, O.; Kertes, E.; Kállai, V.; László, B.; Berta, B.; Taigiszer, M.; Moradi, H.; Mintál, K.; Tóth, A.; Kovács, A.; Lénárd, L.; Karádi, Z.; László, K. The role of intraamygdaloid oxytocin in the regulation of learning-related mechanisms. In: FAMÉ 2023 – Young Pharmacologists' Forum, Mátraháza, Hungary, June 7–9, 2023. In: FAMÉ 2023 Conference Book of Abstracts. p. 56. (oral presentation)

13. Vörös, D.; Kiss, O.; Ollmann, T.; Mintál, K.; Péczely, L.; Zagorácz, O.; Kertes, E.; Kállai, V.; László, B. R.; Berta, B.; Tóth, A.; Lénárd, L.; László, K. Sulpirid blocks the anxiolytic effect of oxytocin in elevated plus maze test. In: Hungarian Neuroscience Doctoral Conference for Undergraduate Students, Graduate Students and Junior Post-Docs, Pécs, Hungary, January 24, 2024. In: Book of Abstracts. p. — (oral presentation)

14. Vörös, D.; Kiss, O.; Lénárd, L.; László, K. Az intraamygdaloid oxitocin magatartási hatásai neurotipikus és autisztikus jeleket mutató patkányokon. In: Pannon Tudományos Nap 2024

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