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RESEARCH ARTICLE

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Sex differences in the glutamate signaling pathway in juvenile rats

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Abstract

Females have been found to be at lower risk for the development of neurodevelopmental disorders than males. The greater neuroprotection in females is mostly due to female sex hormones. Estrogen is hypothesized to provide neuroprotection by suppressing the neuro-excitotoxicity induced by glutamate (Glu). This study was conducted to understand the effect of sex in modulating Glu signaling in juvenile rats. Brain tissue homogenate of 15 Wistar albino rats (9 males, 6 females) weighing 60 to 80 g and aged approximately 28 days was used. Biochemical parameters related to Glu signaling, such as the absolute and relative concentrations of Glu, gamma aminobutyric acid (GABA), and glutamine, as well as glutamate transporter 1 (GLT1), glutamine synthetase (GS), glutaminase (GLN), and glutamate decarboxylase-67 (GAD-67), were measured by ELISA. The data obtained demonstrated that compared with the levels in males, female rats exhibited significantly lower levels of Glu ($p = .001$) and GLN/GS ($p = .021$). The Glu/GABA and Glu/GLT1 ratios as well as the levels of GAD-67 were also lower in female rats, although the difference was not significant. The GLN/GAD-67 ratio ($p = .027$) and levels of GS ($p = .019$) were significantly higher in female rats than in males. Multiple regression analysis confirmed the role of GLN/GS, together with the much higher affinity of GLT1 to Glu, in avoiding excitotoxicity in females. In conclusion, there was a significant difference in Glu signaling between female and male rats. The females exhibited a lower susceptibility to develop Glu-induced excitotoxicity, an etiological mechanism for multiple neurodevelopmental disorders.

KEYWORDS
glutamate signaling, excitotoxicity, glutamine synthetase, glutaminase, glutamate decarboxylase, gamma aminobutyric acid, male, female, autism

1 | BACKGROUND

Imbalanced neurotransmitter levels have been known to contribute to many neurodevelopmental disorders. Glutamate (Glu) excitotoxicity resulting from persistent and uncontrolled neuronal activation usually induces neuron death through multiple mechanisms. First, Glu induces cell death through the overactivation of the N-methyl-D-aspartate receptor that leads to a large Ca^{2+} influx (Choi, 1992). Second, an oxidative pathway involves the breakdown of the Glu-cystine antiporter

Abbreviations: AD, Alzheimer disease; E/I, excitatory/inhibitory; GABA, gamma aminobutyric acid; GAD-67, glutamate decarboxylase-67; Gln, glutamine; GLN, glutaminase; GLT1, glutamate transporter 1; Glu, glutamate; GS, glutamine synthetase; LH, luteinizing hormone; PRL, prolactin.

Significance

Based on the fact that there are sex differences in the susceptibility to and progression of many diseases, identifying the sex-specific factors that protect one sex from a particular disease can be of great interest. This work is an attempt to understand the sex differences in glutamate signaling as an etiological mechanism for many neurological diseases, with special reference to autism, a disorder that more often affects males than females. Studying animals might help to understand the implications of sex differences in translational neuroscience research.

and a remarkable decrease in glutathione levels, which induce the formation of neurotoxic reactive oxygen species (Murphy, Miyamoto, Sastre, Schnaar, & Coyle, 1989; Li, Maher, & Schubert, 1998). Clearance of Glu from the extracellular space via its uptake by various transporters helps to regulate its synaptic levels and hence prevent excitotoxicity. Glutamate transporter 1 (GLT1) in rodents is responsible for over 90% of Glu uptake in the brain (Tanaka et al., 1997).

While Glu regulates excitatory synaptic transmission, gamma aminobutyric acid (GABA) acts as an inhibitory neurotransmitter, and equilibrium between these two neurotransmitters in the brain is critically important for avoiding pathological conditions. GABA-mediated calcium signaling regulates a number of important developmental processes, including cell proliferation, differentiation, synapse maturation, and cell death (Owens & Kriegstein, 2002). Dysfunction of GABAergic signaling early in development leads to a severe excitatory/inhibitory (E/I) imbalance in neuronal circuits, a condition that may account for some of the behavioral deficits observed in patients with neurodevelopmental disorders such as autism (Pizzarelli & Cherubini, 2011). The GABA level and Glu/GABA and glutamine (Gln)/Glu ratios, together with the activity of glutaminase (GLN), glutamine synthetase (GS), and glutamate decarboxylase-67 (GAD-67), are usually used as measures of Glu signaling (Harada et al., 2011; Abu Shmais, Al-Ayadhi, Al-Dbass, & El-Ansary, 2012).

Molecular pathways that contribute to the protection against Glu-induced excitotoxicity in the rat brain are of great interest. The neuroprotective effects of prolactin (PRL) and estrogen as female sex hormones against hippocampal neurodegeneration have been extensively studied (Velíšková, Velisek, Galanopoulou, & Sperber, 2000; Ciriza, Azcoitia, & García-Segura, 2004; Tejadilla, Cerbón, & Morales, 2010; Morales, Lorenson, Walker, & Ramos, 2014). Previous work has shown that pretreatment with PRL diminishes hippocampal neurodegeneration caused by the Glu agonist kainic acid (Tejadilla et al., 2010; Morales et al., 2014) in ovariectomized females.

Furthermore, Conn & Pin and Dingledine et al. have demonstrated that estrogens are neuroprotective against the oxidative pathway of Glu-induced cell death in a mouse hippocampal cell line, HT22 (Conn & Pin, 1997; Dingledine, Borges, Bowie, & Traynelis, 1999). Although estrogens are potent antioxidants (Condorelli et al., 1992; Miller, Romano, & Cotman, 1995) that can prevent oxidative damage in cell culture systems, recent evidence also suggests that estrogen may inhibit Glu-induced excitotoxicity (Silva, Theriault, Mills, Pennefather, & Feeney, 1999). Moreover, estrogen can lower the cytotoxic Ca^{2+} influx induced by Glu in hippocampal cells (Pasti, Volterra, Pozzan, & Carmignoto, 1997; Aronica, Catania, Geurts, Yankaya, & Troost, 2001).

This observation might be related to other studies that reported sex differences in brain tissue Glu (and GABA) content in the neonatal rat, with higher content in males than females (Davis, Ward, Selmanoff, Herbison, & McCarthy, 1999). Bredewold et al. demonstrated that there may be longer-lasting sex differences in Glu release in vivo and subsequently in the ratio of the Glu and GABA concentration (Bredewold, Schiavo, van der Hart, Verreij, & Veenema, 2015).

Females are less susceptible to the development of autistic features than males, and social deficits, as the core symptom in autism, are largely modulated by GABA and Glu neurotransmission (Alessandri, 1992; Moller & Husby, 2000; Jordan, 2003; Bredewold et al., 2015). Therefore, a better understanding of the sex-dependent differences in Glu signaling might be of great importance for understanding why females are less vulnerable to the development of autistic features.

This information initiates our interest to study Glu signaling–related parameters in female and male juvenile rats in an attempt to understand the effect of sex in modulating this signaling.

The rationale for the use of juvenile rats in the present study is firstly due to the fact that the intensity of the neurobiological brake (presented by neurotransmitters, among which are GABA and Glu) on the hypothalamic gonadotrophic-releasing hormone during juvenile development is lower in female rats. Moreover, its effect on gonadotropin secretion is amplified by the negative feedback action of ovarian steroids. Secondly, juvenile rats rather than adult rats are the preferred subjects for studying the effect of sex on Glu excitotoxicity as an etiological mechanism of a neurodevelopmental disorder such as autism.

2 | MATERIALS AND METHODS

2.1 | Animals

The experimental assay of the present study was performed on 15 Wistar albino rats (9 males and 6 females) weighing approximately 60 to 80 g (approximately 28 days old). Animals were obtained from the Center for Laboratory Animals and Experimental Surgery, Department of Zoology, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia, and were raised under standard laboratory conditions with the fertility cycle controlled. They were fed standard diets. Animals were kept individually in separate cages under optimal aeration and temperature with ad libitum access to food and water, with the quantity of food consumed the free choice of each animal.

The experimental protocol was approved by the ethics committee for animal research of King Saud University, Riyadh (4/67/357670). Biochemical parameters representing Glu signaling were measured in the brain homogenates of both male and female juvenile rats.

2.2 | Chemicals

The ELISA kit for Glu was obtained from MyBioSource (catalog no. MBS047402); the kits for GABA, GS, and GLN were obtained from cloud-clone crop (catalog nos. CEA900Ge, SED761Ra, and SEJ026Ra, respectively); the kit for GAD-67 was obtained from ElAab (catalog no. E1258r); and the kit for GLT1 was from MyBiosource (catalog no. MBS2020019).

2.3 | Tissue preparation

The whole brain of both male and female juvenile rats was removed from the skull and dissected into small pieces. The brain tissue (100 mg) was rinsed with 1 × PBS, homogenized in 1 ml of 1 × PBS and stored overnight at −20 °C. After performing two freeze–thaw cycles to break the cell membranes, the homogenates were centrifuged for

TABLE 1 Mean ± *SD* of glutamate signaling–related parameters in male and female rat pups

Parameter	Sex	*N*	Min	Max	Mean ± *SD*
Glu (μg/ml)	Male	7	1,876.20	2,349.42	2,125.35 ± 166.92
	Female	6	1,515.24	1,809.90	1,686.00 ± 120.46**
GABA (pg/ml)	Male	8	973.96	1,652.21	1,400.35 ± 213.74
	Female	6	1,121.81	1,571.50	1,316.83 ± 177.68
Glu/GABA ($\times 10^3$)	Male	7	125.00	249.00	162.78 ± 39.24
	Female	6	107.00	164.00	133.80 ± 25.23
GLT1 (ng/ml)	Male	7	4.20	11.40	6.51 ± 2.47
	Female	6	5.58	7.02	6.27 ± 0.77
Glu/GLT1 ($\times 10^3$)	Male	7	164.00	559.00	358.00 ± 123.78
	Female	6	221.00	310.00	266.75 ± 39.86
GS (pg/ml)	Male	7	996.20	1,789.51	1,476.03 ± 272.22
	Female	6	1,642.49	2,564.19	1,942.24 ± 370.81*
GLN (ng/ml)	Male	7	68.61	108.13	85.61 ± 11.74
	Female	6	79.32	117.46	91.72 ± 15.71
GLN/GS	Male	7	41.51	79.49	59.74 ± 13.02
	Female	6	45.80	48.29	47.34 ± 0.99*
GAD-67 (ng/ml)	Male	7	1.40	3.12	2.10 ± 0.55
	Female	6	1.32	1.89	1.65 ± 0.25
GLN/GAD-67	Male	7	31.11	64.18	42.76 ± 10.22
	Female	6	49.26	63.48	57.18 ± 6.78*

* $p < .05$, ** $p < .01$.

TABLE 2 Multiple regression using the stepwise method for Glu (μg/ml) as a dependent variable in males

				Model	
Predictor variable	Beta	*p*	Adjusted R^2	*F*	*p*
Glu/GABA ($\times 10^3$)	8.258	.001	0.772	72.151	.001
Glu/GABA ($\times 10^3$)	11.815	.001	0.950	200.323	.001
GABA (pg/ml)	1.682	.001			

5 min at 5,000*g* at 2 °C to 8 °C. The supernatant was collected and stored at −80 °C. All biochemical assays were run in duplicate.

2.4 | Protein assays

The protein content of the rat brain preparations was determined by the Bradford method, using bovine serum albumin as the standard (Bradford, 1976).

2.5 | Biochemical assays (ELISA)

All protein markers were assayed using ELISA according to the manufacturer's instruction manual. The volumes of all samples were normalized to ensure that the same amount of total protein was used in the ELISA assay. The sensitivity for various ELISA kits was as follows: Glu assay kit (1.0 μg/ml), GABA assay kit (< 9.25 pg/ml), EAAT2 assay kit (< 0.057 ng/ml), GS assay kit (< 6.2 pg/ml), GLN assay kit (< 0.135 ng/ml), and GAD6-7 assay kit (0.39–25 ng/ml).

2.6 | Statistical analysis

SPSS version 16.0 was used for data analysis, and results were expressed as mean ± *SD*. All statistical comparisons were made by means of Student *t* test. Significant difference was considered at $p < .05$. The smaller number of male animals listed in Table 1 is due to the exclusion of one or two outlier values obtained. A multiple regression analysis was also performed using the same SPSS program. This statistical tool was used to find the correlation between the selected parameters. In this analysis, R^2 describes the proportion of variance in the dependent variables (Glu and GLT1) explained by the variance in the independent variables together, which are sometimes called the predictor variables (GS, GLN, GAD-67, Glu/GABA, and GLN /GS). An

TABLE 3 Multiple regression using the stepwise method for Glu (μg/ml) as a dependent variable in females

				Model	
Predictor variable	**Beta**	***p***	**Adjusted R^2**	***F***	***p***
Glu/GABA ($\times 10^3$)	10.073	.001	0.783	51.384	.001
Glu/GABA ($\times 10^3$)	12.492	.001	0.969	218.551	.001
GABA (pg/ml)	1.641	.001			
Glu/GABA ($\times 10^3$)	11.888	.001	0.978	211.055	.001
GABA (pg/ml)	1.531	.001			
GLN/GS	6.827	.030			

TABLE 4 Multiple regression using the stepwise method for GLT1 (ng/ml) as a dependent variable in males

				Model	
Predictor variable	**Beta**	***p***	**Adjusted R^2**	***F***	***p***
Glu/GLT1 ($\times 10^3$)	−0.015	.001	0.472	19.746	.001
Glu/GLT1 ($\times 10^3$)	−0.022	.001	0.853	62.086	.001
Glu (μg/ml)	0.003	.001			

TABLE 5 Multiple regression using the stepwise method for GLT1 (ng/ml) as a dependent variable in females

				Model	
Predictor variable	**Beta**	***p***	**Adjusted R^2**	***F***	***p***
Glu/GLT1 ($\times 10^3$)	−0.010	.013	0.342	8.270	.013
Glu/GLT1 ($\times 10^3$)	−0.018	.001	0.595	11.297	.002
Glu (μg/ml)	0.002	.011			

R^2 of 1.00 indicates that 100% of the variation in the dependent variable is explained by the independent variables. Conversely, an R^2 of 0.0 indicates the absence of variation in the dependent variable due to the independent variables.

3 | RESULTS

The data of the present study are presented as mean ± *SD*. Table 1 presents the mean ± *SD* of six independent Glu signaling–related parameters in the brain homogenate of male and female juvenile rats. These include measurement of the concentration of Glu (μg/ml), GABA (pg/ml), GLT1 (ng/ml), GS (pg/ml), GLN (ng/ml), and GAD-67 (ng/ml). The mean ± *SD* of the ratio of Glu/GABA, Gln/GLT1, and GLN/GS were also calculated and included in Table 1. Results demonstrate a significantly lower level of Glu ($p = .001$) in female juvenile rat brains than in males. The Glu/GABA ratio as well as the Glu/GLT1 ratio were found to be lower in female rats compared with males, though the values were not statistically significant (Table 1). However, the GLN/GS ratio in female rats was significantly lower ($p = .021$) than that in male rats. The ratio of GLN/GAD-67 was also estimated and was significantly higher ($p = .027$) in female rats than in males. The concentration of GS in the brain cells of females was significantly increased ($p = .019$) compared with that in males. GLN concentration was not significantly different between males and females (Table 1), though, as mentioned before, the GLN/GS ratio was significantly lower in females.

To examine how the various measured independent variables relate to a particular variable selected as the dependent variable, multiple regression analyses were performed. The multiple regression for Glu as the dependent variable in male and female rats is presented in Tables 2 and 3, respectively. Notably, while GABA and the Glu/GABA ratio as predictors or independent variables showed a 95% contribution (adjusted R^2 value of 0.95) in males, a more or less similar percent contribution of GABA and Glu/GABA, as well as GLN/GS, was recorded in females. Tables 4 and 5 show multiple regression analyses using GLT1 as the dependent variable in male and female rats, respectively. Notably, in both sexes, Glu and Glu/GLT1 are shown to be predictive markers of the GLT1 concentration but with a much higher percentage in males than in females (85.3% and 59.5%, respectively).

4 | DISCUSSION

Glu taken up by astrocytes can be converted to Gln, metabolized through the tricarboxylic acid cycle to provide energy, or used in

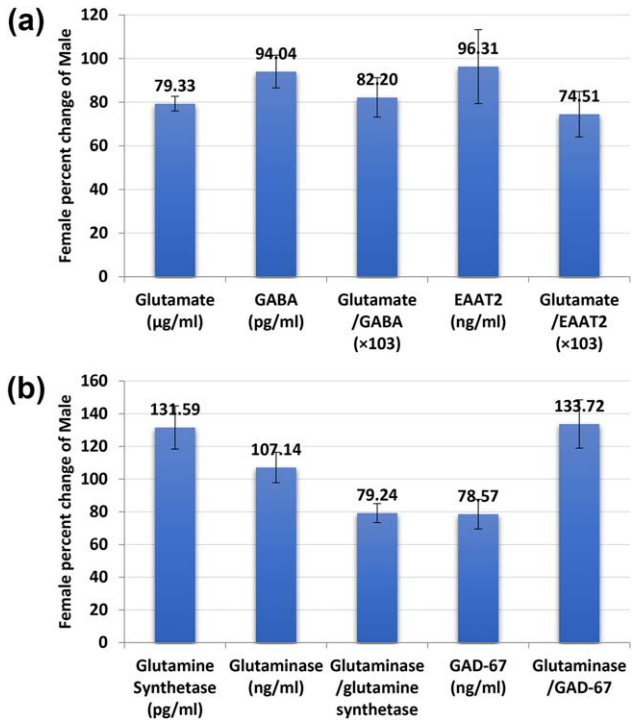

FIGURE 1 (a) Percentage of Glu (μg/ml), GABA (pg/ml), Glu/GABA ($\times 10^3$), GLT1 (ng/ml), and Glu/GLT1 ($\times 10^3$) in females compared with males. (b) Percentage of GS (pg/ml), GLN (ng/ml), GLN/GS, GAD-67 (ng/ml), and GLN/GAD-67 in females compared with males. [Color figure can be viewed at wileyonlinelibrary.com]

protein synthesis. Gln is inactive in the sense that it cannot activate Glu receptors; moreover, it buffers the excitatory effect of Glu Hertz, (2013). The conversion of Glu to Gln is catalyzed by the enzyme GS in an ATP-dependent manner (Marcaggi & Coles, 2001). Thus, GS plays important roles in the brain as well as in other organs. In neurons, Gln that is synthesized in astrocytes is converted back to Glu through the action of GLN. GABA is derived from Glu under the decarboxylation of GAD-67 via Gln-Glu-GABA circulation; thus, changes in the Glu level could affect the GABA content as well (Harada et al., 2011).

Sex hormones have been implicated in neurite outgrowth, synaptogenesis, dendritic branching, myelination, and other important mechanisms of neural plasticity. The trophic effects of estrogen and progesterone as ovarian hormones begin early in brain development and remain throughout life (Weis et al., 2008; Juraska, Sisk, & DonCarlos, 2013). Their actions occur in different regions of the brain that are involved in memory (Liu et al., 2008), emotion (Amin, Epperson, Constable, & Canli, 2006), motor control (Horstink, Strijks, & Dluzen, 2003), and cognition (Berman et al., 1997).

The present study was conducted to understand the effect of gender in modulating Glu signaling in juvenile rats. A significantly lower level of Glu observed in female rats compared with males (Table 1 and figure 1) can be explained on the basis that female sex hormones exert several mechanisms through which they can affect glutamatergic neurotransmission. Impaired regulation of Glu receptors is well documented to result in excessive Glu levels that might lead to Glu excitotoxicity. Based on this phenomenon, the remarkable role of estrogen in the upregulation of Glu receptors (AMPA and NMDA) (Liu et al., 2008; Kramar et al., 2009) can explain the significantly lower level of Glu reported in the present study.

GABA is the most abundant inhibitory neurotransmitter in the brain (Sieghart & Sperk, 2002; Marshall, 2008). $GABA_A$ receptors are responsible for most of the inhibitory GABAergic actions in the brain and are targeted by female sex hormones (Bäckstrom et al., 2011, 2014). While estrogen suppresses GABA inhibitory input (Murphy, Cole, Greenberger, & Segal, 1998), progesterone and its neuroactive metabolites, allopregnanolone and pregnanolone, seem to potentiate GABAergic inhibitory transmission through the activation of $GABA_A$ receptors (van Wingen et al., 2008; Deligiannidis et al., 2013). This suggests that female rats may be less susceptible to Glu excitotoxicity through a more balanced E/I neurotransmission ratio. This hypothesis is supported by the Glu/GABA ratio presented in Table 1. A significantly lower Glu/GABA ratio in female than in male rats may be related to their lower vulnerability to the excitotoxic effect of Glu through sex hormone–potentiated GABA inhibitory neurotransmission.

Neuronal Glu transporters that are located on the astrocytes outside of the synaptic cleft are responsible for most of the Glu reuptake and clearance in the brain (Lehre, Levy, Otterson, Storm-Mathisen, & Danbolt, 1995; Furuta, Rothstein, & Martin, 1997). A profound amount of evidence indicates that a reduction or block of these transporters increases the vulnerability of neuronal death by Glu excitotoxicity (Rothstein et al., 1996; Brustovetsky, Purl, Young, Shimizu, & Dubinsky, 2004; Nieoullon, 2009).

Liang et al. demonstrated that the rate of uptake of Glu by GLT-1 was greatly reduced in Alzheimer disease (AD) astrocytes compared with a much higher rate in the control astrocytes (Liang, Valla, Sefidvash-Hockley, Rogers, & Li, 2002). They added that estrogen treatment greatly affected the transporter uptake of Glu through its effect on the affinity of GLT-1 and GLAST to Glu. Estrogen-treated transporters of AD astrocytes were shown to have a lower K_m and higher affinity to Glu than untreated astrocytes. This observation might be supported by the present data in which a lower ratio of Glu/GLT1 was recorded (Table 1) in female rats than in males. A lower ratio might be related not only to the lower Glu level reported but also to the estrogenic effect on the affinity of GLT1 for Glu. Furthermore, the lack of a difference in the level of GLT1 between the sexes in the present study provides more support for this observation.

The GLN/GS ratio in female rats was significantly lower, whereas the GLN/GAD-67 ratio was higher, than in male rats. GS, as the major Gln-forming enzyme, is usually used as a marker of astrocyte maturation. This enzyme plays a critical role in avoiding Glu excitotoxicity through the control of Glu and Gln concentrations. Table 1 also demonstrates the remarkable increase in the GS concentration in the brain cells of females compared with males. This result is supported by previous work by Haghighat (2005) in which GS expression was reported to be significantly higher in C6 glioma cells treated with estrogen than in untreated cells. Estrogen significantly increased the concentration of Gln and GS when measured using fluorometric assay and Western blotting analysis, respectively.

Although Glu is thought to not readily cross the blood-brain barrier (Sheldon & Robinson, 2007), the level of Glu in the blood is positively correlated with the cerebrospinal fluid level of Glu in humans (McGale, Pye, Stonier, Hutchinson, & Aber, 1977; Alfredsson, Wiesel, & Tylec, 1988). In the present study, although the concentration of GLN was not significantly different between males and females (Table 1), the significantly lower GLN/GS ratio in females can be easily related to the higher vulnerability of males for the development of Glu excitotoxicity compared with that of females. This hypothesis can find support in the previous study by Shimmura et al. (2011) in which plasma Glu and Gln levels were shown to serve as a diagnostic tool for the early detection of autism, especially normal IQ autism. These findings indicate that glutamatergic abnormalities in the brain may be associated with the pathobiology of autism. In the present study, the remarkable increase in Glu together with the concomitant decrease in the GS concentration and GLN/GS ratio in males compared with those in females might be related to the fact that autism is 4-fold more common in males than in females.

A nonsignificantly lower level of GAD-67 in females than males, together with a significantly higher GLN/GAD-67 ratio observed in the present study, is in agreement with previous work by Searles et al. (2000). They had found that GAD-67 expression and GABA turnover were reported to be higher in male rats than females in specific populations of hypothalamic GABAergic neurons (Searles et al., 2000). The lack of a significant difference in GAD-67 in females compared with that in males observed in the present study can be attributed to the difference in the studied brain regions. An early study also demonstrated that GABAergic neurons are steroid sensitive and that GAD mRNA levels were inversely related to the serum luteinizing hormone (LH) level, suggesting a role for GABAergic neurons in the mechanism controlling gonadal steroid negative feedback on LH secretion. These observations can be easily related to the previously reported GABAergic/glutamatergic imbalance in certain neurodevelopmental disorders such as autism (El-Ansary & Al-Ayadhi, 2014) and might also be related to the remarkably higher incidence of autism in males than females.

Multiple regression analysis confirmed the role of GN/GS, together with the much higher affinity of GLT1 to Glu, in avoiding excitotoxicity in females.

These observations provide support for the role of GS in buffering Glu through the formation of Gln and can explain the reduced susceptibility of female rats to develop Glu excitotoxicity as an etiological mechanism in autistic patients and rodent models of autism (Abu Shmais et al., 2012; El-Ansary & Al-Ayadhi, 2014).

The observation that Glu and Glu/GLT1 is much lower in female rats compared to males can support the hypothesis that the affinity of GLT1 for Glu is affected by sex.

4.1 | Limitations and future perspectives

Although our findings support the role of sex differences in Glu excitotoxicity as an etiological mechanism involved in many neurological disorders, we are aware of the limitations of the current study. First, the number of animals is small, and replicating these data with a larger number of animals is necessary. Second, studying the effect of administering female sex hormones on the measured parameters is greatly recommended. Moreover, based on the obtained data, further studies related to the effect of sex on oxidative stress, neuroinflammation, and mitochondrial dysfunction as three important pathophysiological mechanisms related to many brain diseases are highly recommended.

5 | CONCLUSIONS

This study demonstrates significant differences in Glu signaling in females compared with males, which can be related to the reduced susceptibility of females to develop Glu excitotoxicity as an etiological mechanism of multiple neurodevelopmental disorders, with special reference to autism as a disorder that is remarkably more common in males than females. While females demonstrate a much higher buffering power of Glu (lower GLN/GS), males demonstrate a higher vulnerability to the development of excitotoxicity (higher Glu with lower affinity to GLT1).

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

E.A. performed the practical experiments; S.A. codrafted the manuscript; A.E. suggested the topic and drafted the manuscript.

ETHICS APPROVAL

This work was approved by the ethics committee for animal research of King Saud University, Riyadh (No: 4/67/357670).

AVAILABILITY OF DATA AND MATERIAL

Please contact the corresponding author for data requests.

FUNDING

Research Centre, King Saud University, Riyadh, Saudi Arabia.

DATA ACCESSIBILITY

Data are available as a supplementary Excel sheet document.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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