The response to sedative doses of propofol and dexmedetomidine in a prenatal valproate autistic rat model

Soha A. Elmorsy[a], Ghada F. Soliman[a], Laila A. Rashed[b], Hamed Elgendy[c,d,e]

Departments of [a]Medical Pharmacology, [b]Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo, [c]Department of Anesthesia, Assiut University Hospitals, Assiut, Egypt, [d]Department of Anesthesia, HAMAD Medical Corporation, [e]Department of Anesthesia, Weill Cornell Medicine, Doha, Qatar

Correspondence to Soha A. Elmorsy, MD, PhD, Department of Pharmacology, Faculty of Medicine, Cairo University, Cairo, 12411, Egypt. Tel: +201066260082; e-mail: sohaelmorsy@gmail.com

Received 21 November 2017
Accepted 25 November 2017

Kasr Al Ainy Medical Journal
2018, 24:32–39

Introduction
Autism is a challenging neurodevelopmental disorder. Previous clinical observations point to altered sedation requirements of autistic children. The current study aims to test this observation experimentally and to explore its possible mechanisms.
Materials and methods
Eight adult female Sprague Dawley rats were randomly divided into two groups of four each: four were injected with intraperitoneal sodium valproate on the gestational day 12.5 and four were injected with saline. On postnatal day, 28 delivered male rats were subjected to an open-field test to confirm autistic features. Then each rat was injected intraperitoneally with a single dose of propofol (50 mg/kg) or dexmedetomidine (0.2 mg/kg). Time to loss of righting reflex (LORR) and time to return of righting reflex were recorded, and on the next day, all rats were re-sedated and their electroencephalographies were recorded. Rats were killed, and hippocampal $GABA_A$ receptor gene expression and glutamate *N*-methyl-d-aspartate receptor gene expression were assessed.
Results
Autistic rats showed significantly longer time to LORR and significantly shorter time to return of righting reflex as compared with controls for both dexmedetomidine and propofol treatments (median time to LORR: 12.0 versus 5.0 for dexmedetomidine and 22.0 and 8.0 for propofol; $P<0.05$). Electroencephalograph showed a slow, high-amplitude wave pattern 2 min after LORR in control rats, whereas autistic ones showed a high-frequency low-amplitude awake pattern. Hippocampal $GABA_A$ receptor gene expression was significantly less in autistic rats, and *N*-methyl-d-aspartate receptor gene expression was significantly more.
Conclusion
The results of the current study confirm the clinical observations of increased anesthetic sedative requirements with autism and propose a mechanism for it.

Keywords:
autism, dexmedetomidine, GABA, glutamate, loss of righting reflex, propofol

Kasr Al Ainy Med J 24:32–39

1687-4625

Introduction

Genetic and environmental factors interplay to result in autism and may alter the neuronal response to pharmacologic agents [1]. Autistic individuals are repeatedly subjected to investigations that require the use of sedatives. Observational studies suggest that autistic children have different anesthetic requirements from normal ones [2]. Observational studies, however, are vulnerable to confounding. Propofol and dex medetomidine are commonly used for short procedure and pediatric sedation and anesthesia, and each has its own distinct mechanism of action [3,4]. The present work aims to study the sedative requirements of propofol and dexmedetomidine in a rat model of autism.

Materials and methods

The study design and the animal model

Animal experiments were carried out in accordance with the ethical guidelines adopted by the Faculty of Medicine, Cairo University. Figure 1 summarizes the study design. To study the influence of autism on sedative requirements, we had to induce autism-like disorder in rat pups by injecting their mothers with valproate during conception. To secure the required number of rat pups, eight adult female Sprague Dawley rats were obtained from the animal house of the National Ophthalmology Institute and were allowed to mate. The first gestational day was recorded as the first day spermatozoa were noted in the vagina. On the 12th day of gestation, the pregnant females were randomly divided into two groups of four females each: the first group was injected subcutaneously with sodium valproate (Depakine 100 mg/ml; Sanofi

 DOI: 10.4103/kamj.kamj_37_17

Aventis, France) in a dose of 600 mg/kg dissolved in 0.9% saline for a concentration of 100 mg/ml [5]. The other four females provided rat pups for the control group and were injected subcutaneously with saline. After delivery, rat pups were allowed to stay with their mothers until weaning. Only male pups were used for the experiments.

Examination for autistic features and study assessments

All pups were weighed weekly to compare weight gain between the two groups (autistic group and control group). For confirmation of autistic features in the 'autism model group', eye opening was assessed daily starting from the seventh postnatal day. Rats were weaned at 21 days of age, and they were transferred to the Medical Pharmacology Department, Cairo University, and housed in groups of 2–4 in standard housing cages with free access to food and water. To prevent any possible litter effect, rat pups from each individual mother were independently randomized into the propofol or the dexmedetomidine groups. 'Open-field test' was done on postnatal day 28; each animal was placed in the center of an open-field square chamber (60×60 cm, 30 cm in height) [6]. Lines divided the bottom of the chamber into 16 squares. We assessed the number of grooming and rearing movements, and the number of squares traversed by each rat in a 3 min period were counted [7]. Video recording was used to verify counting by an observer blinded to the group assignments [8].

Loss and return of righting reflex

To determine the sedative requirements, each rat was injected intraperitoneally with a single 50 mg/kg dose of propofol (provive 1%; Claris Lifesciences Limited, India) or 0.2 mg/kg dexmedetomidine hydrochloride (Precedex, 100 μg/ml; Hospira Inc., Lake Forest, Illinois, USA). Doses were guided by the work of Yuan *et al.* [9] for propofol and Doze *et al.* [10] for dexmedetomidine, but the choice of the doses actually used was based on preliminary pilot experiments. Time to loss of righting reflex (LORR) was measured as the time in minutes from the intraperitoneal injection to the complete LORR from prone position. Time to return of righting reflex (RORR) was measured as the time from LORR to the time of spontaneous return to supine position [11].

Electroencephalography recording

After return of righting from the first sedative dose, rats were housed overnight, and on the next day, 1.5x the sedative dose was given intraperitoneally. Two minutes after LORR, electrodes were placed under the rat scalp: the positive electrode on one side of the head, the negative one on the other, and a reference electrode at the back of the head. Electroencephalographic recording was captured on PowerLab (ADInstruments ML866; 430-0820, Dunedin, New Zealand). Shielded, low weight, flexible cables connected the electrodes to the input electroencephalograph (EEG) dual bioamplifier (ADInstruments ML408; DBS337, Dunedin, New Zealand). EEG was recorded on a single channel. The recorded EEG was visually inspected, and the pattern was compared between control and autistic model animals to judge on the depth of sedation.

Hippocampus extraction

Hippocampal extraction was done according to the method described by Spijker [12]. Decapitation of animals was done at the end of experiments, and the heads were kept in the refrigerator for 10 min. Through a midline incision in the skin and the bone, the parietal bones were removed, and the brain was exposed, extracted, and transferred to a metal plate placed on ice. The brain was cut in its midline, and the two brain halves were gently separated. The hind brain, midbrain, and the olfactory part were removed from each half and then it was placed such that its medial surface faced upward. Using a spatula, the brain tissue was held, and the tip of another spatula was inserted close to the corpus callosum, thalamus, and the striatum. This allowed the hippocampus to be seen easily. The spatula was put on the medial surface of the hippocampus that was separated easily from the cortex which had a different color. The hippocampus was immediately placed in an Eppendorf and stored at −70.0°C till the time of PCR analysis.

Quantitative analysis of gene expression of $GABA_A$ and glutamate *N*-methyl-d-aspartate receptors by real-time PCR

Total RNA extraction

Total RNA was extracted from the hippocampal tissue homogenate using SV Total RNA Isolation System (Promega, Madison, Wisconsin, USA) according to manufacturer's instructions. The RNA concentration and purity were measured with an ultraviolet spectrophotometer.

Complementary DNA synthesis

The cDNA was synthesized from 1 μg of RNA using SuperScript III First-Strand Synthesis System as described in the manufacturer's protocol (#K1621; Fermentas, Waltham, Massachusetts, USA). Overall, 1 μg of total RNA was mixed with 50 μM oligo (dT) 20, 50 ng/μl random primers, and 10 mM dNTP mix

in a total volume of 10 µl. The mixture was incubated at 56°C for 5 min, and then it was placed on ice for 3 min. The reverse transcriptase master mix containing 2 µl of 10× RT buffer, 4 µl of 25 mM $MgCl_2$, 2 µl of 0.1 M DTT, and 1 µl of SuperScript III RT (200 U/µl) was added to it, and then the mixture was incubated at 25°C for 10 min followed by 50 min at 50°C.

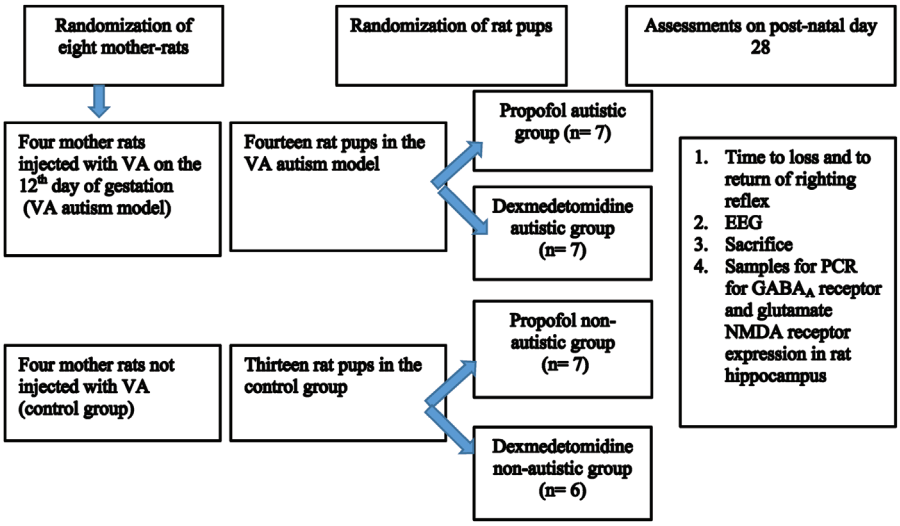
Real-time quantitative PCR

Real-time PCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne, USA). The reaction contained SYBR Green Master Mix (Applied Biosystems) and gene-specific primer pairs (Table 1) and was designed with Gene Runner Software (Hasting Software Inc., Hasting, New York, USA) from RNA sequences from the gene bank. All primer sets had a calculated annealing temperature of 60°C. Quantitative RT-PCR was performed in a 25 µl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer, and 2 µl of cDNA. Amplification conditions were as follows: 2 min at 50°, 10 min at 95°, 40 cycles of denaturation for 15 s, and annealing/extension at 60° for 10 min. Data from real-time assays were calculated using the v1·7 sequence detection software from PE Biosystems (Foster City, California, USA). Relative gene expression of studied gene mRNA was calculated using the comparative Ct method. All values were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) which was used as the control housekeeping gene and reported as fold change over background levels detected in the study groups.

Statistical method

Analysis was done on SPSS (IBM Inc., USA) version 21.0. Sample size estimation was based on an assumed mean difference between autistic and control rats in the time to LORR or RORR of 10 min and a SD of 6 at a two-sided α of 0.05 and β of 0.2. Numeric data were examined for normality and were presented as median and quartiles. Groups were compared using the nonparametric Mann–Whitney test. Because grooming was done either once or not at all in all rats, it was compared as a categorical variable by χ^2-test. To test for the correlation of TOR with the measured $GABA_A$ and *N*-methyl-d-aspartate (NMDA) receptors gene expressions, Spearman's rank correlation was used.

Figure 1



Study design and assessments. EEG, electroencephalography; NMDA, *N*-methyl-d-aspartate; VA, valproic acid.

Table 1 The primer sequence of the studied genes

	Primer sequence
$GABA_A$ receptor	Forward primer: 5′-AATTGAATTCCGCTACCAT-3′
	Reverse primer: 5′-AATTAAGCTTTCCTGTGAC-3′
Glutamate receptor (NMDA)	Forward primer: 5′-CTTGACAAGATGGGCAACAG-3′
	Reverse primer: 5′-TCCTTCTCTCCGAGGATCAA-3′
GAPDH	Forward primer :5′-TGAACGGGAAGCTCACTGG-3′
	Reverse primer: 5′-TCCACCACCCTGTTGCTGTA-3′

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NMDA, *N*-methyl-d-aspartate.

Results

Autistic features

Table 2 shows a comparison between the rats exposed to valproate injection and those with no exposure. There was no statistically significant difference in weight between the two groups at birth or at the time of weaning. However, the weight of autistic rats was numerically lower. Rats with induced autism exhibited delayed eye opening. On open-field examination, rats with autism traversed significantly less squares in the open-field test (P=0.032). They also showed significantly less rearing movements as compared with control rats (P=0.016). Grooming behavior showed no significant difference between the two groups.

Response to sedatives

As shown in Table 3, rats with induced autism treated with propofol or with dexmedetomidine needed a significantly longer time period to lose their righting reflex in comparison with that needed by the control group rats. Autistic model rats also needed significantly less time to RORR.

Electroencephalography pattern

Figure 2 shows EEG tracings taken 2 min after LORR in response to dexmedetomidine or propofol injection in control and autistic rats. EEGs from control rats show the pattern of high amplitude that is usually encountered with sedation, whereas EEGs from autistic rats show low-amplitude high-frequency tracing, going more with an awake state.

PCR of hippocampal $GABA_A$ receptors and glutamate receptors gene expression

Table 4 shows that for dexmedetomidine-treated and for propofol-treated rats, hippocampi from autistic rats had significantly lower $GABA_A$ receptor and significantly higher glutamate receptor gene expression (P=0.001). Within the control and the autistic groups, there was neither a statistically significant difference between $GABA_A$ gene expression in the hippocampi of rats treated with propofol and those treated with dexmedetomidine nor a significant difference in the glutamate receptor gene expression (P>0.05).

Correlation between time to loss of righting reflex and hippocampal $GABA_A$ gene expression and glutamate receptor gene expression

Scatter plots in Fig. 3 show a negative relation between the time to LORR and the hippocampal gene expression of $GABA_A$ receptors with both propofol and dexmedetomidine and a positive relation between glutamate receptor gene expression and time to LORR. Table 5 shows the results of statistical test of correlation confirming the observations on the scatter plots. Statistically significant negative correlation exists between the hippocampal $GABA_A$ receptor gene expression and the time to LORR, and a statistically significant positive correlation exists between the hippocampal

Table 2 Features of autism compared between the control group and the autistic model groups

	Median (quartiles)		*P* value
	Control group (*n*=13)	Autism model group (*n*=14)	
Birth weight (g)	4.2 (3.9–4.5)	3.6 (2.9–5.0)	0.276
Weight at 28 weeks (g)	47.8 (44.0–51.8)	32.5 (29.0–53.5)	0.171
Number of squares traversed	6.0 (4.0–11.0)	3.0 (2.0–7.0)	0.032*
Rearing movements	6.0 (4.0–13.0)	3.0 (2.0–6.0)	0.016*
Grooming movements	Number of rats performing grooming movements/total in the group		
	4/13	2/14	0.385

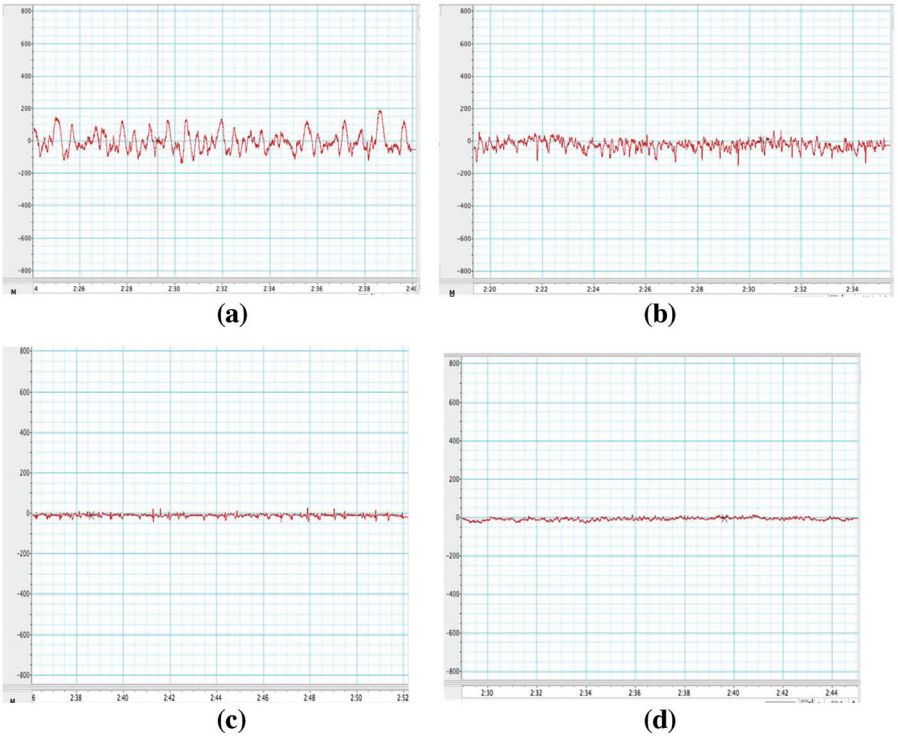
*Statistically significant.

Table 3 Measures of response to sedatives in control and autistic rats

Tested drug	Parameter assessed	Median (quartiles)		*P* value
		Control group	Autism model group	
Dexmedetomidine (*n*=7 in the autistic group and *n*=6 in the control group)	Time to LORR (min)	5.0 (4.0–7.0)	12.0 (11.0–13.0)	0.002*
	Time to RORR (min)	30.0 (20.0–44.0)	10.0 (9.0–28.0)	0.037*
Propofol (*n*=7 in each group)	Time to LORR (min)	8.0 (5.0–11.0)	22.0 (15.0–25.0)	0.001*
	Time to RORR (min)	37.0 (25.0–67.0)	12.0 (10.0–40.0)	0.020*

LORR, loss of righting reflex; RORR, Return of righting reflex; *Statistically significant.

Figure 2



Electroencephalography tracings 2 min after loss of righting reflex from control rats given dexmedetomidine (a), control rats given propofol (b), autistic model rats given dexmedetomidine (c), and autistic model rats given propofol (d).

Table 4 Results of $GABA_A$ receptor and glutamate receptor gene expression in hippocampi of control and autistic rats

Tested drug	Gene expression	Median (quartiles)		*P* value
		Control group	Autism model group	
Dexmedetomidine (*n*=7 in the autistic group and *n*=6 in the nonautistic group)	$GABA_A$ receptor	1.01 (1.00–1.05)	0.21 (0.16–0.76)	0.001*
	Glutamate NMDA receptor	1.01 (1.00–1.06)	4.5 (2.10–13.80)	0.001*
Propofol (*n*=7 in each group)	$GABA_A$ receptor	1.01 (1.00–1.03)	0.22 (0.18–0.73)	0.001*
	Glutamate NMDA receptor	1.00 (1.00–1.07)	4.80 (3.90– 7.20)	0.001*

NMDA, *N*-methyl-d-aspartate; *Statistically significant.

glutamate receptor gene expression and the time to LORR ($P<0.05$).

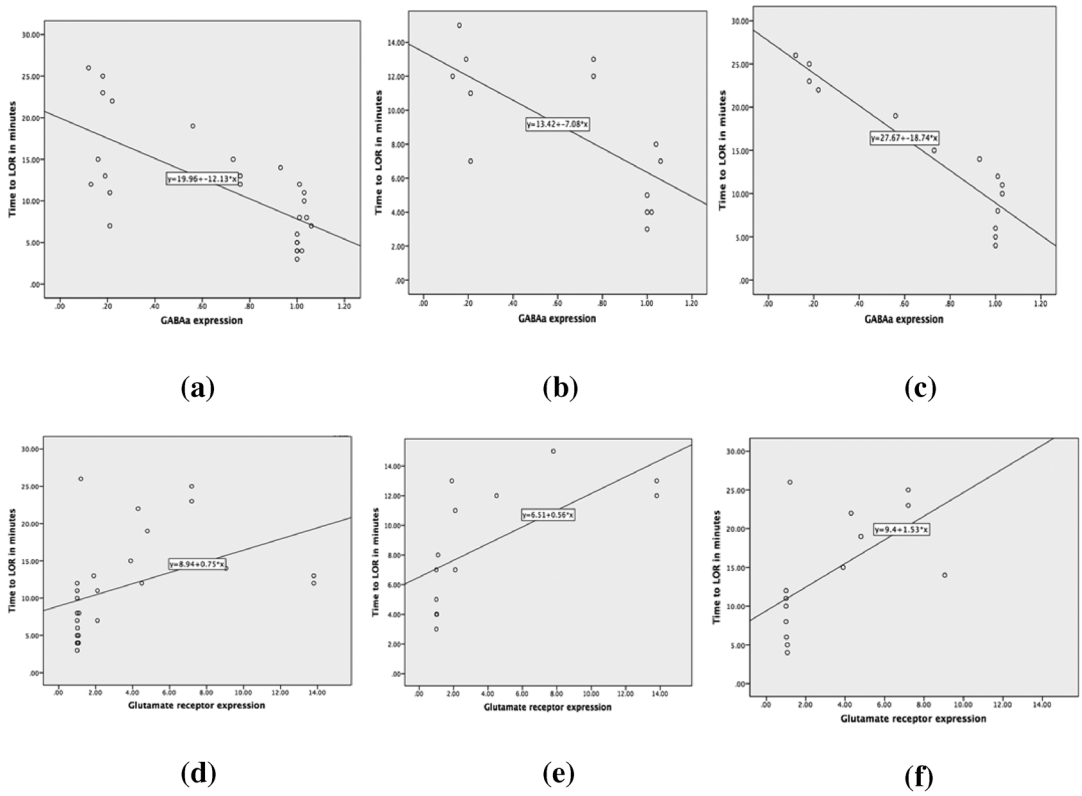
Discussion

The present study investigates the response to sedatives in an autistic rat model that has been repeatedly used by several investigators to mimic the features of autism by injection of valproate in pregnant rats to induce the disease in the offspring. Clinical observational studies have also suggested an association between autism and intrauterine exposure to valproate [13]. Autistic model rats showed a decreased exploration of the surrounding in the open-field test and significantly less rearing movements as previously observed by Pierce and Courchesne [7] and by Imanaka *et al.* [14]. This supports the validity of the model used in the current study in mimicking the autistic features reported in humans.

Time to LORR in rats is the equivalent of anesthetic-induced sedation in humans [15]. Autistic rats given both sedatives needed significantly longer time to achieve LORR and recovered their reflex more quickly than did the control rats. These results confirm previous clinical observations [2].

Electrical brain activity is known to pass through certain changes during sleep. During the awake state, EEG mainly takes the form of low-amplitude high-frequency waves, and during sleep, it takes the form of slow, high-amplitude waves [16]. Pilge *et al.* [17] reported similar EEG patterns during sedation.

Figure 3



$GABA_A$ receptor expression and time to loss of righting reflex in all rats (a), dexmedetomidine rats (b), and propofol rats (c), and glutamate receptor expression and time to loss of righting reflex in all rats (d), dexmedetomidine rats (e), and propofol rats (f). LORR, loss of righting.

Table 5 Results of correlation of hippocampal $GABA_A$ receptor and glutamate *N*-methyl-d-aspartate receptor gene expression to the time to loss of righting reflex

Drug groups	$GABA_A$ receptor gene expression		NMDA receptor gene expression	
	Correlation coefficient (*r*)	*P* value	Correlation coefficient (*r*)	*P* value
All animals (*n*=27)	–0.656	<0.001*	0.678	<0.001*
Dexmedetomidine (*n*=13)	–0.664	0.013*	0.819	0.001*
Propofol (*n* 14)	–0.789	0.001*	0.621	0.018*

NMDA, *N*-methyl-d-aspartate; *Statistically significant.

In the present study, control rats showed typical low-frequency, high-amplitude waves 2 min after LORR. EEGs of autistic rats showed more or less an awake pattern even after LORR. Awake patterns during sedation have been shown by MacIver and Bland [18] in rats under isoflurane anesthesia several seconds before rats recovered their righting reflex. When Cusmano and Mong [19] exposed rats prenatally to valproate then remotely monitored their EEGs after weaning, rats spent significantly more time awake and less time in nonrapid eye movement sleep. Observations in the current study indicate that the depth of attained sedation in autistic rats was still shallow 2 min after LORR. This confirms the diminished response of autistic rats to sedation in comparison with that of normal rats.

The hippocampus is an important channel for signals coming to the higher brain and plays a major role in modulation of behavior and in establishing long-term memory, which is important for learning and social behavior [20]. Several experimental studies linked neuronal or biochemical defects in the hippocampus to the existence of autistic features in animals [21]. Some clinical and experimental studies suggested a role of $GABA_A$ receptors [22] and glutamate [23] abnormalities in the pathophysiology of autism. In this study, we determined the level of gene expression of $GABA_A$ and glutamate NMDA receptors in the hippocampus. Animals with induced autism had lower gene expression of $GABA_A$ receptors and had higher hippocampal glutamate receptor gene expression. $GABA_A$ receptors are ion channel receptors that generate fast inhibitory postsynaptic potentials and

lead to inhibition of various brain functions, and they are targets for several CNS inhibitory drugs. Glutamate is a strong excitatory brain neurotransmitter that acts on both ionotropic [NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainic acid (KA) receptors] and metabotropic receptors [24]. Thus, both changes point to the brain being more on the excitatory side in autistic animals, and this can explain their need for a longer time to achieve LORR. In fact, an imbalance of excitatory/inhibitory neurotransmission has always been a recognized feature of autism [25].

Disorders of $GABA_A$ receptors and neurons have been shown in different models of autism. In a gene knock-out model in mice, Sgadò *et al*. [26] detected alterations in the gene expression and morphology of GABAminergic neurons in the cortex. Impairment of GABAminergic transmission was shown electrophysiologically by Banerjee *et al*. [27] in rats exposed parenterally to valproate. In the current study, time to LORR correlated significantly and positively with hippocampal glutamate receptor gene expression and negatively with hippocampal $GABA_A$ receptor gene expression. Alterations in time to LORR were noted with both propofol and dexmedetomidine, even though they have distinct mechanisms of action. Propofol binds to the ligand-gated $GABA_A$ ion channel receptors and thus can be affected by the decrease in $GABA_A$ gene expression [28]. The decrease in $GABA_A$ gene expression thus perfectly explains the altered response to propofol. Dexmedetomidine, on the contrary, has a very selective agonistic action on α-2A receptors. By reducing norepinephrine release, dexmedetomidine decreases neuronal activity in the locus coeruleus (LC) of the brain stem and leads to sedation [29]. Delayed response of autistic rats to dexmedetomidine can have two possible explanations. The first is based on the increase in glutamate in the rat brain. Being an excitatory transmitter, glutamate may have resulted in the observed effect with dexmedetomidine. Another explanation is the fact that dexmedetomidine may rely partially and indirectly on GABAmenergic transmission. It was suggested by Nelson *et al*. [30] that inhibition of norepinephrine release from the LC by dexmedetomidine releases inhibitory control over the ventrolateral preoptic nucleus. The later releases GABA and galanin, which lead to more LC inhibition. Ventrolateral preoptic nucleus also inhibits histamine release leading to a hypnotic response. Thus, part of dexmedetomidine action may depend on the GABAminergic system and so may be affected by the $GABA_A$ receptor downregulation.

A limitation of this study is the fact that it was based on a single experimental model of autism, and this model may not have identical pathophysiology to clinical autism. Yet, the model used is a widely acceptable one. So on the whole, the results of this study are in line with previous knowledge about the pathophysiology of autism and encourage us to delve more into its molecular basis. Such knowledge can be used in planning therapy of autism and in managing autistic individuals with intercurrent medical and surgical conditions.

Conclusion

The results of the present study confirm previous clinical observations that autism may alter sedative requirements. Receptor expression analyses have provided some explanation for the observations. It is recommended to plan prospective clinical studies to quantify the different responses to sedatives by autistic children.

Acknowledgements

The study team would like to acknowledge the efforts of Dr Osama Abdelmageed, Associate Consultant of Anesthesia, King Abdullah Medical Complex, Saudi Arabia for his help in logistic matters in the study.

Dr Soha A. Elmorsy contributed to design, literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review; Ghada F. Soliman contributed in literature search, experimental studies, manuscript preparation, manuscript editing, and manuscript review; Laila A. Rashed contributed in experimental studies: PCR analysis and interpretation, manuscript review; Hamed Elgendy contributed to concept, literature search, manuscript editing, and manuscript review.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Schmitz C, Rezaie P. The neuropathology of autism: where do we stand? Neuropathol Appl Neurobiol 2008; 34:4–11.

2 Elgendy H, Ahmed D, Elmorsy S, Aboloyoun A, Banjar A, Youssef T, *et al*. Variation of anesthetic sedation requirements in children undergoing auditory brainstem response (ABR) test: a retrospective cross-sectional Study. J Anesth Clin Res 2016; 7:670.

3 Eilers H, Yost S. General anesthetics. In: Katzung S, Trevor A, editors. Basic and clinical pharmacology. 13th ed. USA: McGraw Hill Education; 2015. 430.

4 Xu J, Jin C, Cui X, Jin Z. Comparison of dexmedetomidine versus propofol for sedation after uvulopalatopharyngoplasty. Med Sci Monit 2015; 22:2125–2133.

5 Dufour-Rainfray D, Vourc'h P, Le Guisquet AM, Garreau L, Ternant D, Bodard S, *et al.* Behavior and serotonergic disorders in rats exposed prenatally to valproate: a model for autism. Neurosci Lett 2010; 470:55–59.

6 Edden RA, Crocetti D, Zhu H, Gilbert DL, Mostofsky SH. Reduced GABA concentration in attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 2012; 69:750–753.

7 Pierce K, Courchesne E. Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. Biol Psychiatry 2001; 49:655–664.

8 Han H, Ma Y, Eun JS, Hong JT, Oh KW. Anxiolytic- like effects of cyclopeptide fraction alkaloids of Zizyphi Spinosi se-men: possible involvement of GAB_{AA} receptors. Biomol Ther 2008; 16:261–269.

9 Yuan D, Su G, Liu Y, Chi X, Feng J, Zhu Q, *et al.* Propofol attenuated liver transplantation-induced acute lung injury via connexin43 gap junction inhibition. J Transl Med 2016; 14:194.

10 Doze V, Chen BX, Li Z, Maze M. Pharmacologic characterization of the receptor mediating the hypnotic action of dexmedetomidine. Acta Vet Scand Suppl 1989; 85:61–64.

11 Chemali1 JJ, Kenny JD, Olutola O, Taylor NE, Kimchi EY, Purdo PL, *et al.* Ageing delays emergence from general anaesthesia in rats by increasing anaesthetic sensitivity in the brain. Br J Anaesth 2015; 115:i58–i65.

12 Spijker S. Dissection of rodent brain regions. In: Ka Wan L, editor. Neuroproteomics, neuromethods. USA: Springer Science+Business Media, LLC; 2011. 13–26.

13 Baxter P. Valproate and folic acid in pregnancy: associations with autism. Dev Med Child Neurol 2014; 56:604.

14 Imanaka A, Morinobu S, Toki S, Yamamoto S, Matsuki A, Kozuru T, *et al.* Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague-Dawley rats. Behav Brain Res 2008; 186:91–97.

15 Zhang Z, Ferretti V, Güntan İ, Moro A, Steinberg EA, Ye Z, *et al.* Neuronal ensembles sufficient for recovery sleep and the sedative actions of $\alpha 2$ adrenergic agonists. Nat Neurosci 2015; 18:553–561.

16 Bhagwandin A, Gravett N, Lyamin OI, Oosthuizen MK, Bennett NC, Siegel JM, *et al.* Sleep and wake in rhythmic versus arrhythmic chronotypes of a microphthalmic species of african mole rat (Fukomys mechowii). Brain Behav Evol 2011; 78:162–183.

17 Pilge S, Jordan D, Kreuzer M, Kochs EF, Schneider G. Burst suppression-MAC and burst suppression-CP(5)(0) as measures of cerebral effects of anaesthetics. Br J Anaesth 2014; 112:1067–1074.

18 MacIver MB, Bland BH. Chaos analysis of EEG during isoflurane-induced loss of righting in rats. Front Syst Neurosci 2014; 16:203.

19 Cusmano DM, Mong JA. In utero exposure to valproic acid changes sleep in juvenile rats: a model for sleep disturbances in autism. Sleep 2014; 37: 1489–1499.

20 Guyton AC, Hall JE. Behavioral and motivational mechanisms of the brain. In: Guyton AC, Hall JE, editors. Textbook of medical physiology. 11th 5 ed. USA: Saunders Elsevier; 2011. 718–719.

21 Auerbach BD, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature 2011; 480: 63–68.

22 Chao HT, 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; 468:263–269.

23 Brown MS, Singel D, Hepburn S, Rojas DC. Increased glutamate concentration in the auditory cortex of persons with autism and first-degree relatives: a (1)H-MRS study. Autism Res 2013; 6:1–10.

24 Gray JA, Nicoll RA. (editors). Introduction to pharmacology of CNS drugs. In: Basic and clinical pharmacology. USA: McGraw Hill Education; 2015. 14 366.

25 Rubenstein JLR, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003; 2:255–267.

26 Sgadò P, Genovesi S, Kalinovsky A, Zunino G, Macchi F, Allegra M. Loss of GABAergic neurons in the hippocampus and cerebral cortex of Engrailed-2 null mutant mice: implications for autism spectrum disorders. Exp Neurol 2013; 247:496–505.

27 Banerjee A, García-Oscos F, Roychowdhury S, Galindo LC, Hall S, Kilgard MP. Impairment of cortical GABAergic synaptic transmission in an environmental rat model of autism. Int J Neuropsychopharmacol 2013; 16:1309–1318.

28 Trapani G, Altomare C, Liso G, Sanna E, Biggio G. Propofol in anesthesia. Mechanism of action, structure-activity relationships, and drug delivery. Curr Med Chem 2000; 7:249–271.

29 Correa-Sales C, Rabin BC, Maze M. A hypnotic response to dexmedetomidine, an alpha 2 agonist, is mediated in the locus coeruleus in rats. Anesthesiology 1992; 76:948–952.

30 Nelson LE, You T, Maze M, Franks NP. Evidence that the mechanism of hypnotic action in dexmedetomidine and muscimol-induced anesthesia converges on the endogenous sleep pathway. Anesthesiology 2001; 95: A1368.