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ORIGINAL ARTICLE

Effects of time-of-day on the concentration of defined excitatory and inhibitory amino acids in the cerebrospinal fluid of rats: a microdialysis study

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Abstract

Amino acid neurotransmitters are responsible for many physiological and pathological processes, and their cerebral concentrations respond to external influences such as the light–dark cycle and to the synthesis, release, and recapture rhythms and form part of the biochemical relationships derived from excitatory-inhibitory (E/I), glutamine–glutamate sum (GLX), glutamatergic processing (glutamine–glutamate ratio) and excitotoxic indexes. The changes in these variables during a 24-h period (1 day) are important because they allow organisms to adapt to external stimuli and form part of physiological processes. Under pathological conditions, the damage produced by acute events may depend on diurnal variations. Therefore, it is important to analyze the extracellular levels of amino acids as well as the above-mentioned indexes over a 24-h period. We focused on determining the cerebrospinal fluid levels of different amino acid neurotransmitters, and the E/I, GLX, glutamatergic processing and excitotoxic indexes, determined by microdialysis over a 24-h cycle. Our results showed significant changes during the 24-h light/dark cycle. Specifically, we found increments in the levels of glutamate (325%), GABA (550%), glutamine (300%), glycine (194%), alanine (304%) and the GLX index (263%) throughout the day, and the maximum levels of glutamate, glutamine, glycine, and alanine were obtained during the last period of the light period. In conclusion, the concentration of some amino acid neurotransmitters and the GLX index show variations depending on the light–dark cycle.

Keywords Amino acid neurotransmitters · Microdialysis · Diurnal variation · E/I index · GLX index · Glutamatergic processing index

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Introduction

Biological processes exhibit rhythmic variations that are synced with geographical cycles and external factors, such as the widely studied light–dark cycle (Garfinkel 1983). In organisms, these biological processes range from the biochemical to physiological and behavioral levels, and one of the aims of these changes is to maintain the internal temporal dynamics of an organism that allow its adaptation to a cyclical environment and thus allow the organism to exhibit efficient responses to external changes; for example, in the brain, these processes can be observed at several levels, including the function of synapses, the release of neurotransmitters, and the expression of receptors, all of which are essential for intercellular communication (Wirz-Justice 1987; Estrada-Rojo et al. 2020). In some processes, such as neuron firing, the period covers only fractions of a second, whereas the period in other processes, such as hibernation or seasonal reproduction, includes cycles that last one to several days, as found in some animals (Groos 1982). Some oscillations occur even in the absence of external synchronizers, and this finding suggests that these processes are regulated by internal clocks, which are known as biological rhythms and include circadian rhythms with a period of approximately 1 day (Wirz-Justice 1987; Jagannath et al. 2017).

These periodic oscillations allow biological systems to exhibit functional advantages. In the brain, these rhythms allow the generation of adduced responses to specific stimuli or as a response to other processes already programmed, such as the sleep–wake cycle, feeding time, initiation of motor activity, and release of hormones (Wirz-Justice 1987). Loss or alteration of these rhythms leads to disorders or makes the brain vulnerable to certain harmful events, whereas their maintenance or recovery leads to decreased suffering (Wirz-Justice 1987; Tapia-Osorio et al. 2013). The most important reservoir of the brain is the ventricle, where the cerebrospinal fluid (CSF) irrigates as an ultrafiltrate of plasma around all principal brain areas (Telano and Baker 2020). The principal functions are nourishment, protection, and waste removal (Damkier et al. 2010) to maintain the homeostasis of the brain interstitial fluid. The removal of waste products of metabolism includes proteins, ions, peroxidation products, other unnecessary molecules, and excess neurotransmitters. The accumulation of these molecules interferes with neural function and may facilitate the presentation of diseases or neurodegenerative processes (Sakka et al. 2011; Spector et al. 2015). For this reason, measurements in this space are fundamental to understanding the normal conditions of the biochemical systems involved in brain function.

Different responses depend on circadian variations; for example, in the injury and neuroprotection process, synaptic amino acid neurotransmission plays an important role, as has been shown for glutamatergic and GABAergic systems, although other systems are also involved. Other parameters, such as the glutamate/γ-aminobutyric acid (GABA) ratio or excitation-inhibition index (E/I) (Dehghani et al. 2016; Gu et al. 2019), the sum of glutamate–glutamine (GLX) (Hall et al. 2015), the glutamine–glutamate ratio (index of glutamatergic processing) (Hashimoto et al. 2005; Hall et al. 2015) and the relationship between glutamate–glycine–GABA (excitotoxic index) (Globus et al. 1991), have not been explored under these physiological conditions. It is worth mentioning that in the context of neurophysiology, the E/I index represents the relationship between excitatory and inhibitory synaptic inputs corresponding to neuronal events in which they participate and provides guidelines for understanding the basic mechanisms through which these systems interact for baseline functioning (Dehghani et al. 2016; Gu et al. 2019). The GLX index represents the biochemical relationship between glutamate and its precursor, glutamine, and may reflect increased or decreased glutamatergic neurotransmission (Levine et al. 2000; Hall et al. 2015). Based on this information, we sought to evaluate the levels of excitatory amino acid neurotransmitters (glutamate and aspartate), inhibitory amino acid neurotransmitters (GABA and glycine), glutamine (the precursor of glutamate, aspartate, and GABA), and alanine as well as the E/I, GLX, glutamatergic processing and excitotoxic indexes in the CSF of rats during a complete light/dark cycle through microdialysis.

Materials and methods

Chemicals

The reagents used for high-performance liquid chromatography (HPLC) analysis (glutamate, aspartate, GABA, glycine, alanine, and glutamine standards) and artificial cerebrospinal fluid (ACSF) were purchased from Sigma Co. (St. Louis, MO, USA). All other reagents were obtained from known commercial sources and exhibited appropriate purity for HPLC. Solutions were prepared using deionized water obtained from a Milli-RQ purifier system from Millipore (Billerica, MA, USA).

Animals

Male Wistar rats ($n = 6$, 280–320 g) bred in house were obtained from Harlan, Mexico, and were used in all experiments. The animals had access to food and water ad libitum and were housed individually in polycarbonate box cages under the following standard environmental conditions:

constant temperature (22–24 °C), relative humidity (45–55%), and a 12-h light/dark cycle (lights on at 8:00 am). All animal experiments were approved by the local Research and Ethics Committee [Protocol 128-2009, Faculty of Medicine, Universidad Nacional Autónoma de México (UNAM); Protocol, 04-2013, National Institute of Pediatrics (INP)] and were conducted according to its guidelines. During the experiments, all efforts were made to minimize animal suffering.

Animal surgery

The rats were anesthetized with a combination of ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.m.). A guide cannula was implanted above the left lateral ventricle (AP, − 0.96; ML, 2.0; DV, 3.4; Paxinos and Watson 1998; see Fig. 1). Stainless-steel screws were threaded into the cranium over the frontal cortex, and the assembly was fixed to the skull with dental acrylic. After surgery, all the animals were allowed to recover for 3 days.

In vivo microdialysis of freely moving animals

On experimental day 4, a microdialysis probe (BAS MD-2200, membrane length = 2.0 mm; PAN. 30 kDa MWCO, OD = 320 µm) was inserted into the guide cannula. The animals were placed in an individual container system for rodents (BASi microdialysis system; Raturn® caging system). The probe was then connected to a microperfusion pump and continuously perfused with ACSF (125 mM NaCl, 2.5 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.5 mM NaH_2PO_4, and 5.0 mM Na_2HPO_4) at a flow rate of 2.0 µL/min. Dialysate collection was initiated after a 120-min stabilization period. For individual subjects, samples were collected every 30 min to 4 h over a 24-h period.

Measurement of extracellular neurotransmitter levels by HPLC

The analysis was performed using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a fluorescence detector operated at an excitation wavelength of 360 nm and an emission wavelength of 450 nm, a binary pump, an automatic injector with a sampler, and a column thermostat. We followed the chromatographic conditions and characteristics described by Henderson et al. (2000) with slight modifications. For HPLC, the microdialysis samples were diluted 1:5 with ACSF, and the mixtures were refrigerated for 10 min and then centrifuged at 14,000 rpm for 6 min. The supernatants were filtered, and 15 µL was transferred to microtube vials, placed in amber vials with screw caps and stored in the refrigerated sampler unit of the HPLC system. The HPLC fluorometric free amino acid detection procedure required OPA (o-phthalaldehyde and 3-mercaptopropionic acid in borate buffer, Agilent Technologies) derivatization and a reversed-phase 3.0-mm column (Zorbax Eclipse AAA, Agilent Technologies) with aqueous solution (0.1 M sodium phosphate dibasic, dissolved in Milli-RQ water) and an aqueous solvent solution (acetonitrile, methanol, Milli-RQ water at 4:4.5:1) at a flow rate of 0.5 mL/min. Within each batch of analyses, a concentration of 1.9 µM of all amino acids was used to prepare the standard solution, and internal control solutions (a normal microdialysis sample and 1.9 µM standard at 1:1) were also included as a reference for the peak height measurement and a quantitative control, respectively.

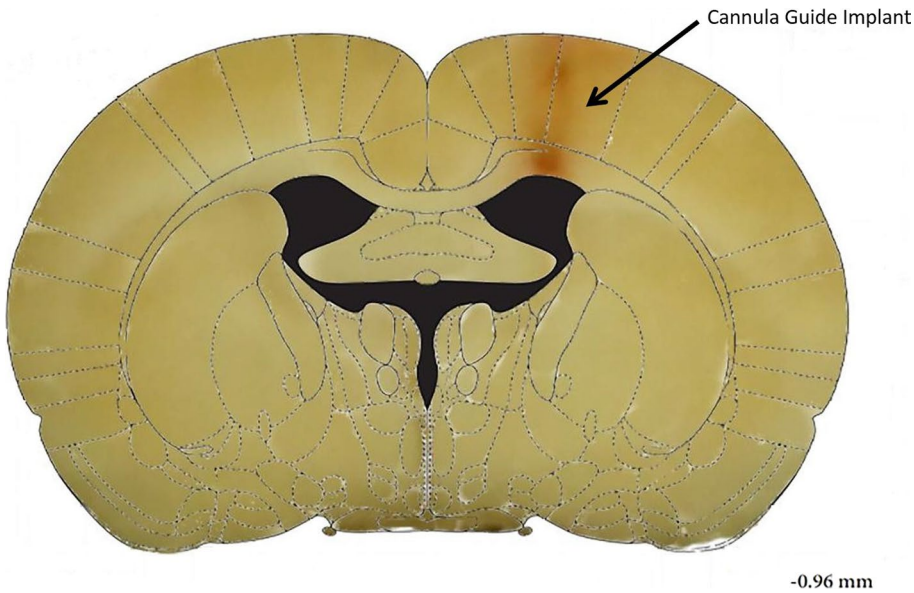
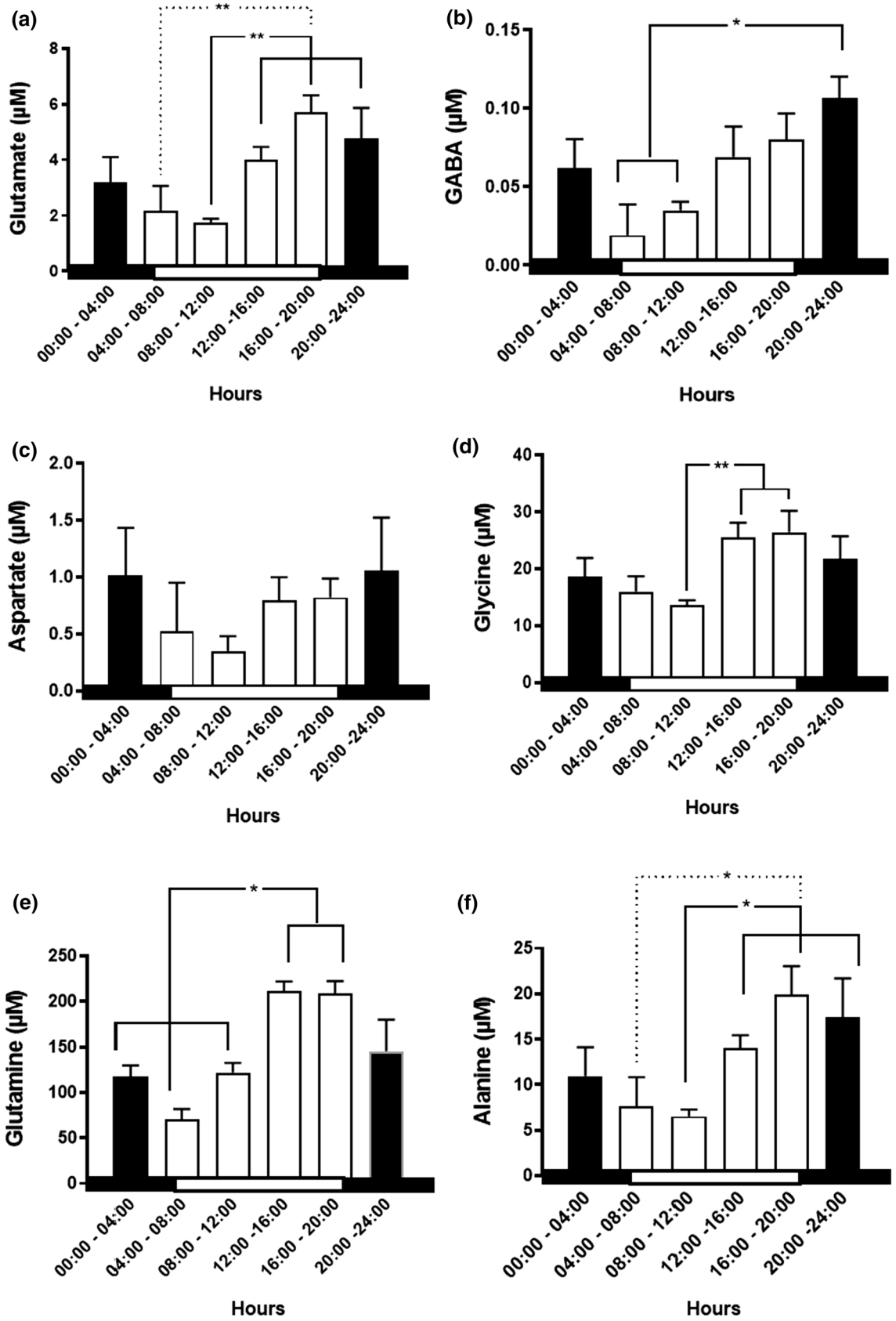


Fig. 1 Brain region implanted with the cannula guide


(a)
Glutamate (μM)
0
2
4
6
8
**
**
00:00 - 04:00
04:00 - 08:00
08:00 - 12:00
12:00 -16:00
16:00 - 20:00
20:00 -24:00
Hours
(b)
GABA (μM)
0.00
0.05
0.10
0.15
*
00:00 - 04:00
04:00 - 08:00
08:00 - 12:00
12:00 -16:00
16:00 - 20:00
20:00 -24:00
Hours
(c)
Aspartate (μM)
0.0
0.5
1.0
1.5
2.0
00:00 - 04:00
04:00 - 08:00
08:00 - 12:00
12:00 -16:00
16:00 - 20:00
20:00 -24:00
Hours
(d)
Glycine (μM)
0
10
20
30
40
**
00:00 - 04:00
04:00 - 08:00
08:00 - 12:00
12:00 -16:00
16:00 - 20:00
20:00 -24:00
Hours
(e)
Glutamine (μM)
0
50
100
150
200
250
*
00:00 - 04:00
04:00 - 08:00
08:00 - 12:00
12:00 -16:00
16:00 - 20:00
20:00 -24:00
Hours
(f)
Alanine (μM)
0
5
10
15
20
25
*
*
00:00 - 04:00
04:00 - 08:00
08:00 - 12:00
12:00 -16:00
16:00 - 20:00
20:00 -24:00
Hours

◂**Fig. 2** CSF levels of different amino acids in the lateral ventricle during the light–dark cycle. The **a** glutamate, **b** GABA, **c** aspartate, **d** glycine, **e** glutamine and **e** alanine levels are presented as the means ± SEMs and were analyzed by one-way ANOVA followed by Tukey's post hoc test. *$P < 0.05$, **$P < 0.001$

Probe placement confirmation

At the end of each experiment, the rats were killed by decapitation under light pentobarbital anesthesia, and their brains were rapidly removed, frozen, and stored at − 70 °C. The location of the guide cannula and probe in the left lateral ventricle was carefully confirmed by histological analysis. The anatomical region analyzed is shown in Fig. 1, which depicts the location of the active portion of the microdialysis probes. Schematic representations of the active portion of the dialysis membranes are depicted as representative coronal sections of the rat brain (according to Paxinos and Watson 1998). Only those animals with correct placement of the guide cannula were included in the statistical analysis.

Statistical analysis

The amino acid neurotransmitter concentrations were calculated by comparing peak areas with those of standard solutions obtained by HPLC, and the E/I and GLX indexes were determined by the arithmetic coefficient and sum between glutamate and GABA (Dehghani et al. 2016; Gu et al. 2019) or glutamate and glutamine (Hall et al. 2015), respectively. The glutamatergic processing index was the glutamine–glutamate ratio (Hall et al. 2015), and the excitotoxic index was arithmetically calculated as glutamate × glycine/GABA (Globus et al. 1991).

Data are presented as the average ± SEM of measurements obtained over 4-h periods for six subjects.

The amino acid neurotransmitter concentration values and the E/I, GLX, glutamatergic processing, and excitotoxic indexes were statistically analyzed by one-way ANOVA followed by post hoc Tukey's test. The analyses were performed using the scientific statistical software GraphPad Prism 5. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

We only included data from animals in which the position of the microdialysis and guide cannula were verified as located in the lateral ventricle on postmortem histological assessment (Fig. 1) and from animals for which we had HPLC information over a 24-h period. The CSF levels of glutamate, GABA, glycine, glutamine, and alanine showed significant variations over the 24-h period and between periods of light and dark, whereas aspartate only tended to decrease during the light period.

Determination of extracellular levels of amino acids

The CSF glutamate concentration exhibited a peak between 16:00 and 20:00 (5.69 µM) and a nadir between 08:00 and 12:00 (1.75 µM), and a difference of 325% was found between the peak and nadir ($P < 0.0001$, one-way ANOVA; $F_{5,64} = 8.903$; Fig. 2a).

We also found significant variations in the CSF GABA levels over a 24-h period, with a peak between 20:00 and 24:00 (0.11 µM), a nadir between 04:00 and 08:00 h (0.02 µM), and a 550% change between the peak and nadir ($P < 0.0036$, one-way ANOVA; $F_{5,64} = 3.923$; Fig. 2b).

The aspartate concentration in the CSF did not show significant variations during the 24-h period; however, we observed a peak between 20:00 and 24:00 (1.06 µM) and a nadir between 08:00 and 12:00 (0.35 µM), and the differences between the peak and nadir was calculated to equal 303% (Fig. 2c).

The CSF glycine concentration showed a peak between 16:00 and 20:00 (26.44 µM) and a nadir between 08:00 and 12:00 (13.59 µM), and a difference of 194% was found between the peak and nadir ($P < 0.0001$, one-way ANOVA; $F_{5,64} = 6.436$; Fig. 2d).

The levels of glutamine in the CSF exhibited a peak between 12:00 and 16:00 h (211.70 µM) and a nadir between 04:00 and 08:00 (70.64 µM), with a 300% change between the peak and nadir ($P < 0.0001$, one-way ANOVA; $F_{5,64} = 11.28$; Fig. 2e).

The alanine levels in the CSF showed a peak between 16:00 and 20:00 (19.90 µM) and a nadir between 08:00 and 12:00 (6.54 µM), and a difference of 304% was detected between the peak and nadir ($P < 0.0001$, one-way ANOVA; $F_{5,62} = 6.938$; Fig. 2f).

According to the release profiles, we observed that the studied neurotransmitters showed variations during the 24-h cycle. The highest concentrations observed in this study were obtained for glutamine, and GABA presented the lowest concentrations. For most amino acids, the lowest values were obtained during the first hours of the light period, and the maximum values were detected in the afternoon and evening.

Evaluation of the E/I, GLX, glutamatergic processing and excitotoxic indexes

The E/I index showed non-significant variations with maximum values between 12:00 and 16:00 (154.60 µM) and minimum values between 20:00 and 24:00 (47.53 µM; Fig. 3a). We also found changes in GLX that were dependent on the phase of the cycle, with a maximum level between 16:00 and

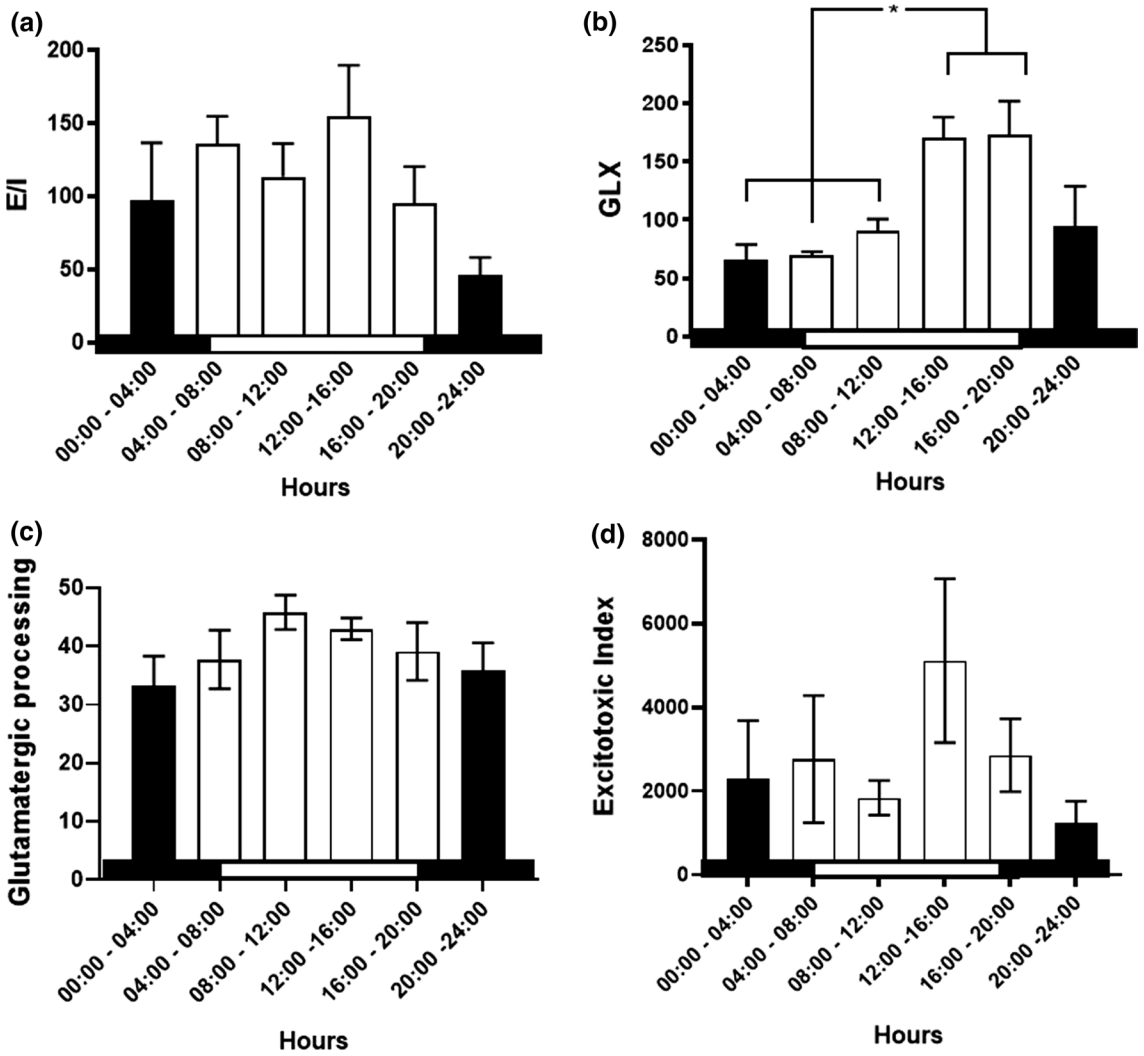


Fig. 3 Indexes during the light–dark cycle. The **a** glutamate/GABA (E/I), **b** sum of glutamate–glutamine (GLX), **c** glutamine–glutamate (glutamatergic processing) and **d** glutamate × glycine/GABA (excitotoxic index) are presented as the means ± SEMs and were analyzed by one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$

20:00 (173.37 µM) and a minimum value between 00:00 and 04:00 (65.71 µM). These variations were statistically significant ($F_{5,64} = 6.685$, $P < 0.0001$; Fig. 3b). The glutamatergic processing index (glutamine/glutamate) exhibited non-significant variations, with maximum and minimum values between 08:00 and 12:00 (45.85 µM) and 00:00 and 04:00 (33.41 µM; Fig. 3c), respectively. The excitotoxic index exhibited non-significant variations with maximum values between 12:00 and 16:00 (5113 µM) and minimum values between 20:00 and 24:00 h (1227 µM; Fig. 3d).

Discussion

Our results showed that the release profiles of the main amino acid neurotransmitters (glutamate, GABA, glycine, glutamine and alanine) and the sum of glutamine–glutamate (GLX) showed variations over 24-h cycles under basal conditions. However, the aspartate levels, E/I, glutamatergic processing (glutamine–glutamate ratio) and excitotoxic indexes, did not show significant changes. These

amino acids were analyzed in the ventricular system, and the most important reservoir of the brain is the ventricle, where the CSF plays an irrigation role as an ultrafiltrate of plasma around all principal brain areas (Telano and Baker 2020). Its principal functions are nourishment, protection, and removal of the waste products of metabolism (including proteins, ions, peroxidation products, other unnecessary molecules, and excess neurotransmitters) to maintain the homeostasis of the brain interstitial fluid (Damkier et al. 2010; Sakka et al. 2011; Spector et al. 2015). The ventricle is utilized as an access point to medication delivery routes and also allows access in the presence of intraventricular pathology (Mortazavi et al. 2014). Although the measurement of the CSF content can be used to detect various pathologies (Bondoli et al. 1981; Greenamyre and Young 1989), very few studies have monitored the profiles of amino acid levels under basal conditions (Wardlaw et al. 2014). In other studies, these levels have been measured in normal patients, but only acute measurements have been obtained as a baseline for comparison with patients with traumatic brain injury (TBI) (Baker et al. 1993). Nonetheless, an analysis of the amino acid levels in the CSF is clinically relevant in several diseases (Rodan et al. 2015; Batllori et al. 2016). Such analyses can allow us to better diagnose, administer amino acid therapy, or determine the processes that are activated when administering certain receptor blockers, as needed (Moreno-Medinilla et al. 2016).

In addition, measurements in this space are fundamental for understanding the normal conditions of the biochemical systems involved in brain function, and the extracellular levels of amino acid neurotransmitters, such as their accumulation in the CSF, could reflect their rate of secretion or degradation, which could in turn exhibit a diurnal rhythm. Neurotransmitters are involved in many functions in the brain; for example, glycine and glutamic acid changes in the CSF of human patients are related to regulation of the energy balance (Wardlaw et al. 2014). In contrast, changes in amino acids in both the plasma and CSF are related to diurnal changes in hormones, such as leptin and pro-opiomelanocortin (POMC), which reinforces the notion that the rhythms of their levels regulate the energetic balance of the brain and surely the rest of the body (Xie et al. 2013; Wardlaw et al. 2014). The foregoing can be key in studies that show differences in the extracellular concentrations during light–dark cycles depending on the brain area (Leenaars et al. 2018). In 2018, Leenaars et al. (2018) performed a systematic review of the literature regarding the diurnal variations of various amino acids in different brain areas measured by microdialysis. These researchers found that in nocturnal rodents, aspartate, glutamate, and histamine are generally higher in the dark than during the light period. No rhythmicity has been found for glutamine, and the GABA results were too inconsistent to allow any generalizations. In contrast, the glycine data are insufficient. However, these researchers did not analyze the CSF levels because this type of study is extremely rare.

The main biological systems exhibit periodic oscillations that confer positive functional advantages to these organisms. For instance, in the brain, these rhythms allow the generation of adduced responses to specific stimuli or as a response to processes already programmed, such as the sleep–wake cycle, feeding time, initiation of motor activity, and hormone release (Wirz-Justice 1987). Loss or alteration of these rhythms leads to disorders or makes the brain vulnerable to certain harmful events, whereas its maintenance or recovery leads to decreased damage (Wirz-Justice 1987; Tapia-Osorio et al. 2013).

Our data show that the levels of the main amino acid neurotransmitters in the rat CSF, glutamate, glycine, glutamine and alanine, show variations over the 24-h period constituting a day, with considerable increases during light hours, significant peaks between 12:00 and 20:00, and a tendency to decrease between 04:00 and 12:00. This variation is different from that found for GABA, which tended to decrease between 04:00 and 08:00 and increase between 20:00 and 24:00. Although other studies have reported that some amino acids are present at high levels at night or do not show changes, it is important to note that these studies were performed in specific brain structures (Choma et al. 1979; Honma et al. 1996; de Prado et al. 2000; Duvilanski et al. 2003; Castañeda et al. 2004; Meng et al. 2015; Leenaars et al. 2018), and our data reflect the global changes observed in the cerebral ventricle. The precise regulation of the CSF allows us to consider that changes in its composition represent an alteration in brain physiology (Telano and Baker 2020).

Our microdialysis results showed that the glutamate, glycine, and glutamine levels increase until reaching a peak at approximately 20:00. These findings do not coincide with the results reported by Castañeda et al. (2004), de Prado et al. (2000), and Meng et al. (2015) in rat striatum or Honma et al. (1996) in rat SCN. They found that the extracellular levels of glutamate are higher during the dark phase. Nevertheless, these studies were not performed in the CSF. However, our results for glutamate, alanine, and glycine coincide with the values reported by Wardlaw et al. (2014) in human CFS, despite the difference between the activity-rest phases of both species. Our results correlate with those obtained with other experiments performed by our group because we documented that the recovery of rats subjected to TBI depends on the time-of-day at which the intervention took place, which also correlates with the motor cortex expression of NMDA receptors (Estrada-Rojo et al. 2018). Individuals who experience brain damage during hours of darkness exhibit improved recovery, which coincides with

a lower expression of NMDA receptors during hours of darkness among individuals who do not experience damage. Thus, the processes that sensitize NMDA receptors by affecting the glutamate levels could contribute to modulating the excitotoxic effect that arises after trauma (Suleiman 2005).

Our glutamine findings are different from those reported by Castañeda et al. (2004) and de Prado et al. (2000), who showed that glutamine does not depend on the circadian rhythm. But they coincide with the values reported by Wardlaw et al. (2014) for humans.

We found that GABA tended to decrease at the beginning of the light phase (04:00–12:00) and subsequently increase during the dark period from 20:00 to 24:00. These data agree with those obtained in other studies that found higher extracellular levels of GABA during the dark phase (de Prado et al. 2000) and with reports of variations in the GABA concentration depending on the light/dark cycle, which showed that the expression of the GABAA receptor β1 subunit in the SCN, retina, and median eminence of hamsters show maximal levels at midnight (Naum et al. 2001). These variations could be related to the study model because Perlow et al. (1979) described that the GABA concentrations in the CSF were higher during the light period in primates (Perlow et al. 1979). Currently, there is strong evidence showing that GABA may regulate the transmission of the circadian output of the SCN and thereby changes the neural excitability. GABA is also involved in synaptic plasticity, for example, in the retinohypothalamic tract, and thus needs to be synthesized and released in a circadian manner (Ono et al. 2018).

It is important to note that glycine and alanine exhibit a release profile similar to that of glutamate, and this behavior could be explained by the role of glycine as an NMDA receptor coagonist (Bowery and Smart 2006) similar to alanine (L-alanine is a weak agonist of NMDA receptors; Curtis and Watkins 1960; Kleckner and Dingledine 1988). At the synaptic level, glycine fluctuates according to both the degree of activation of the synapse and its efflux from astrocytes associated with excitatory terminals. It is important to emphasize that the release of glycine from both neurons and astrocytes depends on the concentration of calcium and the temperature (Harsing and Matyus 2013). However, for this release to occur, the glycine site of NMDA receptors must be saturated, and the interstitial concentration of glycine must be sufficient (Dietrich et al. 1996). Therefore, the profile of the glycine levels reported obtained in our study is significant because it shows that these fluctuations are indeed needed to be able to saturate NMDA receptors and contribute to the excitability of the nervous system (Wood et al. 1993; Wallis et al. 1994). This biochemical process would be similar to that observed with alanine.

Our results show that glutamine also follows a diurnal release pattern, with increased values during light hours. In the central nervous system (CNS), glutamine is the main precursor of both the excitatory amino acid glutamate and the inhibitory neurotransmitter GABA. However, glutamine has also been associated with neuroprotective processes in experiments performed on hippocampal slices without oxygen and glucose (Dal-Cim et al. 2016). Glutamine-containing diets have been used in rehabilitation processes for patients who underwent TBI (Scrimgeour and Condlin 2014). Moreover, in vitro studies have shown that the downregulation of glutamine synthetase activity affects processes such as excitotoxicity and neuroinflammation, which are events that occur in many neurological disorders (Jayakumar and Norenberg 2016). Again, the diurnal release profile of glutamine, as shown in our data, indirectly demonstrates that the activity of glutamine synthetase could also follow a diurnal rhythm. However, this result is different from that described by Castañeda et al. (2004) and de Prado et al. (2000), who reported that glutamine does not show differences depending on the circadian rhythm in particular brain areas.

In contrast, the GLX index exhibited a significant increase from the light to dark periods: the lowest levels were observed from 00:00 to 08:00 and increases to the highest level were detected from 16:00 to 20:00. In the case of E/I, glutamatergic processing and excitotoxic indexes showed bidirectional tendencies without significant differences. Both E/I and the excitotoxic index showed mild increases from 04:00 to 08:00, presented a high level from 12:00 to 16:00 and decreased during the other time periods studied, with the lowest level being detected from 20:00 to 24:00, and the glutamatergic processing index showed high levels during the light period. Our findings also help explain why excitotoxicity, such as that observed after ischemia or TBI, varies depending on the time of occurrence (Vinall et al. 2000; Martinez-Vargas et al. 2006, 2013). Other studies using murine models have demonstrated that these indexes can change in different pathological conditions. As described in male Wistar rats, similar increases in the excitotoxic index during ischemia have been observed in the striatum and thalamus (Globus et al. 1991). The increases in the E/I index (glutamate/GABA) in the hypothalamus and glutamatergic processing index (glutamine/glutamate) in the frontal cortex indicate a neurochemical imbalance in male Wistar rats (Franco-Pérez et al. 2020). Studies in humans have confirmed the associations among glutamate levels, intracranial pressure, and outcome in children with a diagnosis of severe head injury (SCG, 8 or less) prior to ventilation but failed to corroborate the correlation between excitatory and structural amino acid levels in adult patients, and these findings provide evidence of nonspecific leakage of amino acids through damaged cell membranes. The role of glutamine in glutamate homeostasis is an important consideration, and estimation of the extracellular glutamine–glutamate ratio

(glutamatergic processing index) may have prognostic value in these cases and could provide evidence of links to clinical outcome (Richards et al. 2003).

In addition to the experimental evidence from studies conducted with male schizophrenic patients who experienced a first episode and have not received any previous treatment and healthy males of the same age, the mean glutamine–glutamate ratio in the CFS obtained for each patient during the entire microdialysis period revealed a significant difference between these two groups. An increase in the extracellular glutamine–glutamate ratio is associated with a more favorable outcome (Hashimoto et al. 2005). Other studies have found that measurements of Glutamine (Gln) and Glutamate (Glu) from healthy participants who performed a strange auditory task were not a particularly useful index of synaptic glutamatergic measurement. However, the Gln–Glu ratio (glutamatergic processing index) is more specific as a synaptic measure because it reflects the relative amounts of metabolites. Given that Gln synthesis in glial cells and Glu synthesis in neurons allow observation of the Gln–Glu ratio, this result is a potentially useful index for quantifying glial–neuronal interactions and the balance of glutamatergic metabolites. Therefore, elevations and reductions in this index may reflect increases and decreases in Glu neurotransmission, respectively (Hall et al. 2015). These findings offer a novel outlook, suggesting therapeutic windows of opportunity for the treatment of various neurological processes. Further research will allow us to better understand which pathways or mechanisms are involved in the modulation of the extracellular release of the amino acids studied in circadian cycles (absence and presence of light), the impacts these systems have at the pre- and postsynaptic levels, and the possible interactions with other neurotransmission systems under these experimental conditions.

Conclusions

Our results show that the main amino acids with neurotransmitter or neuromodulator functions within the CNS display diurnal rhythms. These findings offer new research perspectives suggesting that the functionality of these amino acids depends on time, which, in turn, indicates that the motor or cognitive processes that depend on these neurotransmitters could also vary with time.

Understanding the diurnal profiles of the levels of these amino acids would shed light on therapeutic options for the treatment of various neurological disorders. Furthermore, previously unsuccessful pharmacological treatments could be reconsidered in light of our findings to determine whether the time of administration and the dose could be adjusted to obtain different effects.

Moreover, the pathways through which the extracellular release of these amino acids is modulated must consider the light–dark cycles, and the roles of circadian markers in these systems are not yet accurately understood.

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Availability of data and materials Data and further information about methods section are available from the corresponding author.

Declarations

Conflict of interest The authors declare they have no financial interests.

Ethics approval All animal procedures were strictly performed following the National Institutes of Health Guideline for the Care and Use of Laboratory Animals, the local guidelines on the ethical use of animals drafted by Mexico's Federal Ministry of Health Ministry, and the Mexican Official Standard NOM-062-ZOO-1999 and are part of project 04-2013, which was approved by Research Boards of the National Institute of Pediatrics (NIP), Mexico City, registered at the Office for Human Research Protection of the NIH (http://ohrp.cit.nih.gov/search/search.aspx) with number IRB00008064, and approved by the NIP, Committee of Laboratory Animal Use and Care, and the local Research and Ethics Committee of the Faculty of Medicine, Universidad Nacional Autónoma de México (UNAM) (Protocol 128-2009).

Consent to participants This research involved animals.

Consent for publication Not applicable in this study.

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