

## BEHAVIORAL AND ELECTROENCEPHALOGRAPHIC MANIFESTATIONS OF THIOACETAMIDE-INDUCED ENCEPHALOPATHY: POSSIBLE MECHANISMS OF NEUROTOXIC EFFECTS

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**Abstract** - Although there is still no ideal experimental model of hepatic encephalopathy, thioacetamide is widely used for the induction of acute and chronic liver failure. Thioacetamide exerts hepatotoxic effects through the formation of toxic metabolites in hepatocytes, oxidative stress and calcium mobilization. An ideal experimental model of hepatic encephalopathy should have similar behavioral and electroencephalographic manifestations as human encephalopathy. Thioacetamide induces motor manifestations in a dose-dependent manner. Milder forms of thioacetamide-induced encephalopathy are associated with an increase in relative alpha power, while more severe forms are followed by a flattening of the electroencephalogram. Liver failure-induced hyperammonemia has a pivotal role in the neurotoxic effects of thioacetamide. Hyperammonemia induces brain edema, alterations in neurotransmission, oxidative stress, mitochondrial dysfunction and neuronal death. The aim of this article is to review the behavioral and electroencephalographic manifestations of thioacetamide-induced encephalopathy, as well as to summarize potential mechanisms involved in thioacetamide neurotoxicity.

**Key words:** Behavioral manifestations, electroencephalography, hepatic encephalopathy, hyperammonemia, thioacetamide

### INTRODUCTION

Thioacetamide (TAA) is a sulfur-containing compound that may be used in agriculture, as well as in rubber and paper industries and metallurgy. TAA is known to exert hepatotoxic, neurotoxic and carcinogenic effects (Albrecht et al., 1990; Hilgier et al., 1991; Norton et al., 1997; Uehara et al., 2008). The hepatotoxic effects of TAA are mediated primarily by the covalent binding of TAA metabolites to macromolecules in hepatocytes (Porter et al., 1979; Dyroff and Neal, 1981). TAA is metabolized in the liver by cytochrome P450-dependent monooxygenase to thioacetamide-S-oxide (Hunter et al., 1977; Porter et al., 1979; Mori and Aoyama, 1983). In the next step,

thioacetamide-S-oxide is converted into acetamide and polar products that bind irreversibly to various macromolecules in hepatocytes (Hunter et al., 1977). Additional mechanisms of the hepatotoxic effects of TAA include the mobilization of calcium from intracellular stores (Diez-Fernández et al., 1996) and oxidative stress (Yogalakshmi et al., 2010). TAA was found to induce lipid peroxidation in the liver, associated with a reduction in catalase activity and reduced glutathione (GSH) level (Sanz et al., 1998). All of these mechanisms contribute to the acute bridging necrosis and apoptosis of hepatocytes after administration of 300 mg/kg TAA. Higher doses (900 mg/kg) provoke extensive inflammation with hemorrhages that lead to the development of acute liver

failure (Mladenović et al., 2012b). In addition, TAA was observed to induce regenerative changes in the liver, evident as an increase in mitotic index. Mitoses increase at 24 h of TAA intoxication and reach a peak at 72 h in adult rats (Sanz et al., 1998). This effect may contribute to cirrhosis development in chronic TAA exposure (Méndez et al., 2008a; 2008b).

The hepatotoxic effects of TAA have been used for the induction of hepatic encephalopathy (HE) in various animal species (Norton et al., 1997; Miranda et al., 2010). HE represents the neuropsychiatric syndrome that appears as a result of acute or chronic liver failure (Gammal and Jones, 1989). The pathogenesis of HE is not completely clear and for the purpose of further investigation, there is a need for to establish the best possible model of this disorder. However, there is still no ideal model of HE that can be used for the investigation of all aspects of pathogenesis in HE. An ideal model should fulfill certain criteria, including reversibility, reproducibility of the experiment, the possibility for investigation of therapeutical procedures on the model and a correlation between the behavioral and electroencephalographic (EEG) manifestations of HE in the model and in humans (Terblanche and Hickman, 1991). This correlation is very important since behavioral and EEG manifestations represent a key method for diagnosis of HE (Amodio et al., 2006).

The aim of this review is to summarize the behavioral and electroencephalographic manifestations of TAA-induced encephalopathy, to compare them with manifestations of human HE, as well as to describe potential mechanisms involved in the pathogenesis of this encephalopathy.

#### *Behavioral and neurological manifestations of TAA-induced encephalopathy*

TAA induces a wide diversity of behavioral changes that are dependent on the animal species used in the experiment and the duration of exposure. The first descriptions of the behavioral changes after TAA administration were obtained from the study of Norton et al. (1997) in Sprague-Dawley rats. According to

these authors, a dose of 1200 mg/kg induced a decline in motor activity with progression to coma 64 h after treatment. Mladenović et al., (2012b) showed that Wistar rats appear to be more sensitive to TAA-induced encephalopathy and that a lower dose of TAA (900 mg/kg) may induce coma in this strain within 24 h after treatment. In addition, TAA induces behavioral and neurological changes in a dose-dependent manner. The general motor activity and exploratory behavior in Wistar rats were decreased after the administration of 600 mg/kg TAA, and a further decline in the motor performance of the rats was evident after the administration of 900 mg/kg TAA. Similar findings were obtained from analysis of headshake, auditory startle reflex, equilibrium and placement test. Corneal, withdrawal, grasping and righting reflex are markedly diminished even 2 h after TAA treatment with a dose of 900 mg/kg (Mladenović et al., 2012b). TAA also induces encephalopathy in C57BL/6 mice, as evaluated by the screening test battery called SHIRPA (Miranda et al., 2010). This battery consists of a wide range of tests, organized in five functional categories: reflex and sensory function, autonomous function, muscle tone and strength, motor behavior and neuropsychiatric state (Rogers et al., 1997). This battery has proved to be efficient in indentifying subtle behavioral changes in HE. Reflex and sensory function, motor behavior and neuropsychiatric state were diminished in C57BL/6 mice receiving 600 mg/kg of TAA. These behavioral and neurological changes were associated with an increase in serum aminotransferases activity (Miranda et al., 2010). Sabra mice were found to be even more sensitive to the neurotoxic effects of TAA, since a dose of 200 mg/kg of TAA induced encephalopathy in this mice strain (Avraham et al., 2006). These findings indicate that TAA in an appropriate dose may be used for the induction of the behavioral manifestations of acute liver failure in mice and rats.

The neurotoxic effects of TAA were found to be sex-dependent. The latency to all four stages of TAA-induced HE and survival were significantly longer in female rats than in male rats (Norton et al., 1997). In addition, gross motor activity and exploratory movements, as well as grasping, righting and placement

reflex were decreased earlier in the course of HE in male rats than in female rats (Norton et al., 1997). These sex-related differences in the behavioral effects of TAA may be explained by sex-dependent variations in fatty acid and TAA metabolism (Kamataki et al., 1986; Hagve et al., 1988; Nozu et al., 1992). Cytochrome P-450 that metabolizes TAA to toxic products has a different activity in male and female rats, thus contributing to more powerful hepatotoxic effects of TAA in males (Kamataki et al., 1986).

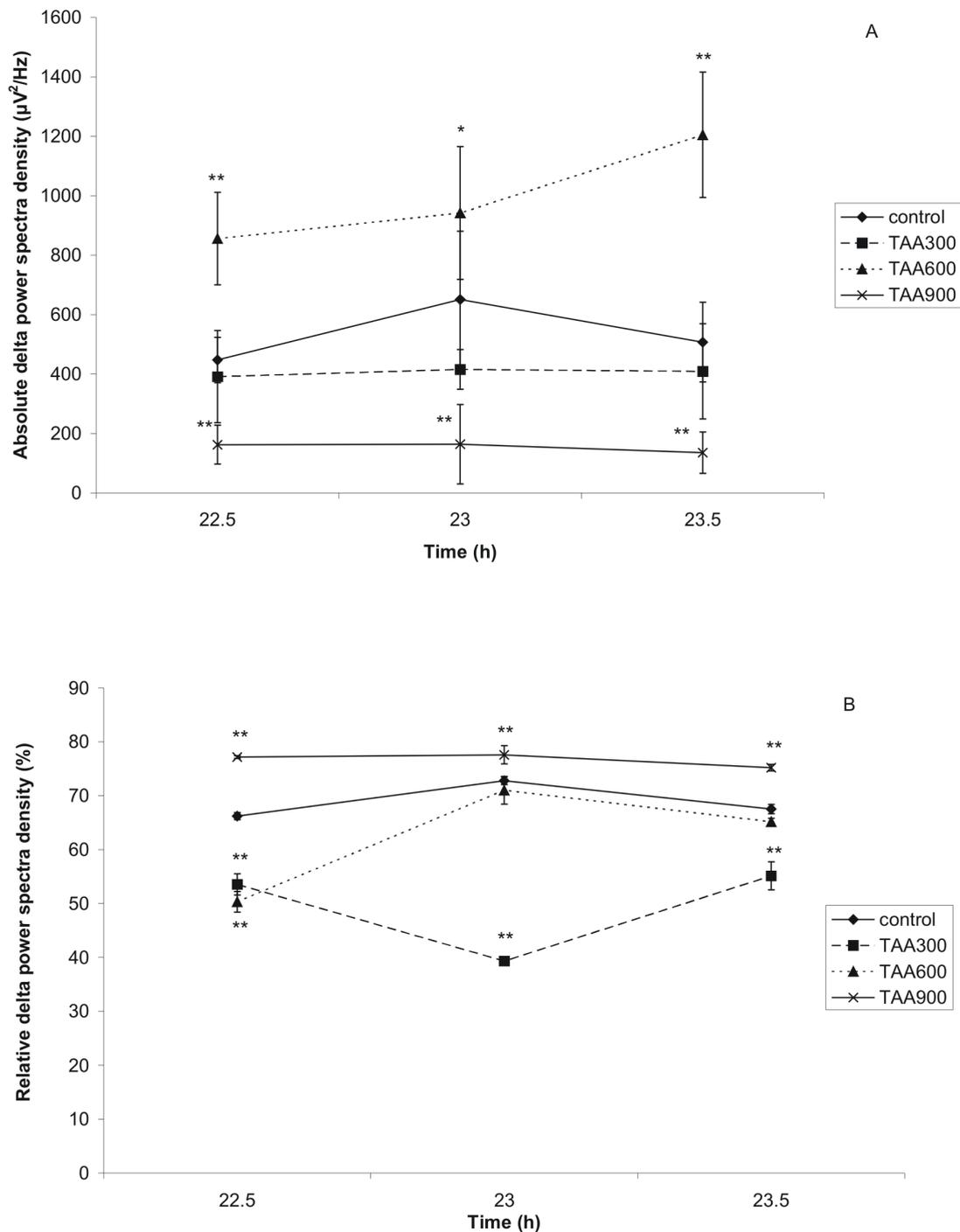
Furthermore, the sensitivity of animals to toxic effects of TAA is also modulated by co-morbidity. Type 2 diabetic rats were shown to be more sensitive to the hepatotoxic effects of TAA than normal rats due to inhibited cell division and compromised tissue repair (Sawant et al., 2006).

In contrast to acute HE, TAA has seldom been used for the induction of chronic HE (Méndez et al., 2008a, 2008b). HE in human cirrhosis may be classified as minimal, episodic or persistent. Minimal HE is characterized by cognitive impairment that is not obvious in the standard neurological exam, but can be detected by neuropsychological and neurophysiological tests. Episodic HE is manifested by acute changes in mental state with or without recognized precipitating factors, while chronic HE encompasses chronic cognitive or motor manifestations that impact negatively on social and occupational activities (Córdoba, 2011). These manifestations may even cause dependency because of dementia, paraplegia or signs similar to Parkinsonism. Common manifestations of chronic HE in humans include bradykinesia and hypokinesia; it is frequently associated with rigidity, hyperreflexia and flapping tremor, altered sleep-waking cycle, changes in personality, impairment of attention and of memory and cognitive function (Albrecht and Jones, 1999; Cauli et al., 2009). Although chronic TAA exposure was found to induce liver cirrhosis in rats, various investigations did not find changes in the motor performance of rats that is evident in human HE (Méndez et al., 2008a; 2008b). On the other hand, chronic TAA administration was found to reduce the ability to form spatial memory maps in rats (Méndez et al., 2008a;

2008b). Similar results with an impairment of learning were found in other models of hyperammonemia (Méndez et al., 2008b; Aguilar et al., 2000). In addition, chronic HE was found to be associated with impaired cognitive flexibility (Wesierska et al., 2006). Learning disturbances in liver cirrhosis and hyperammonemia are also evident in human cirrhosis and may be explained by the impairment of long-term potentiation in the hippocampus and glutamate-NO-cyclic guanosine monophosphate (cGMP) pathway (Muñoz et al., 2000; Monfort et al., 2004; Rodrigo et al., 2005).

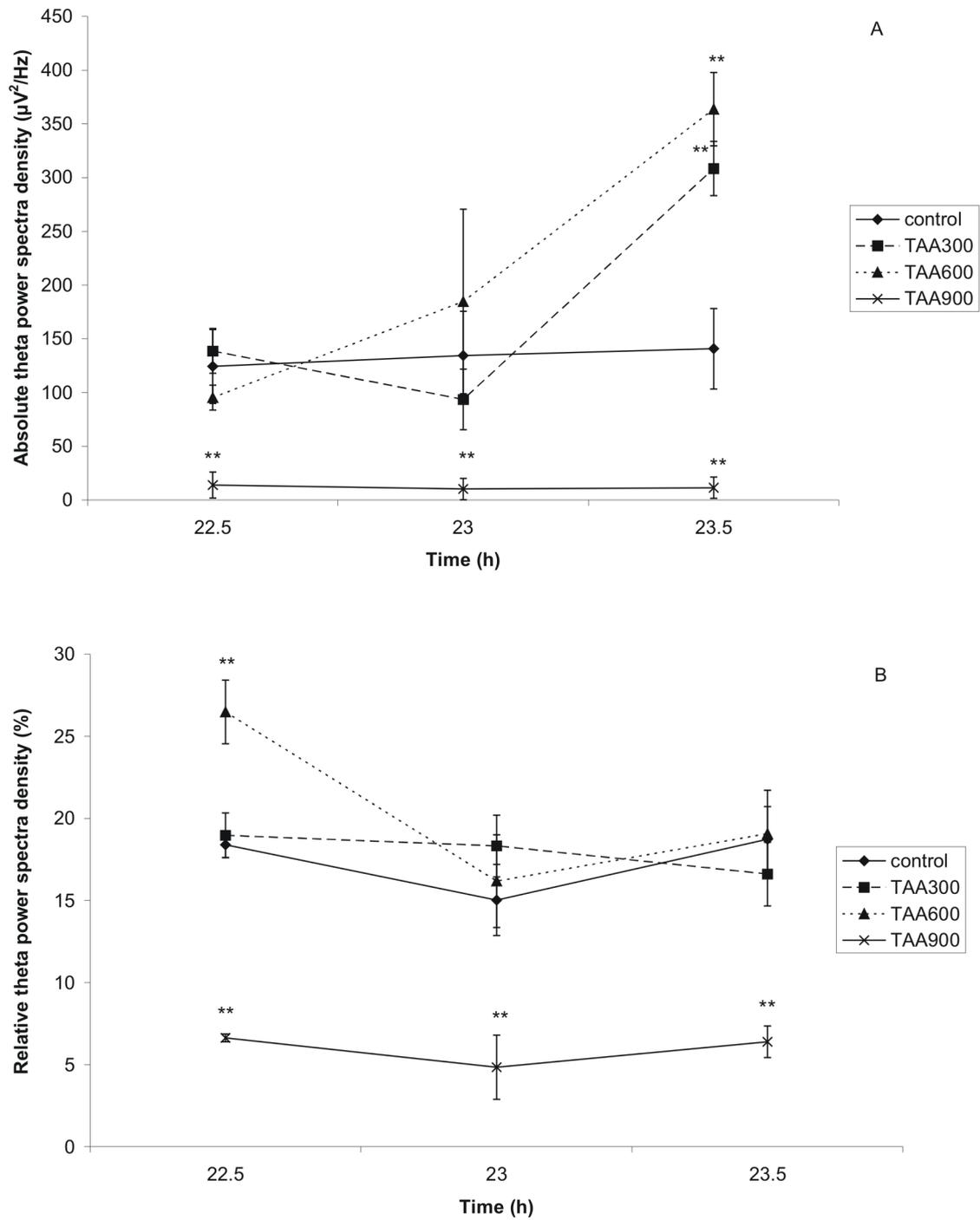
#### *Electroencephalographic manifestations of TAA-induced encephalopathy*

Behavioral changes induced by TAA are also followed by electroencephalographic (EEG) manifestations that correlate with the changes in human HE (Mladenović et al., 2012b). Spectral analysis remains a reliable method for EEG analysis in TAA-induced encephalopathy, since our studies have shown that this method has a diagnostic and prognostic value (Mladenović et al., 2012b). TAA induces EEG changes in a dose-dependent manner. TAA in doses of 300 and 600 mg/kg induces a significant increase in the mean power spectra density in Wistar rats, thus reflecting an increase in EEG voltage. In contrast, a higher dose (900 mg/kg) induces a decrease in mean EEG voltage (Mladenović et al., 2012b). Additionally, TAA has different effects on various EEG bands. Lower doses (e.g. 300 mg/kg) provoke an increase in relative alpha power and a decrease in relative delta power, with a simultaneous increase in voltage in beta, alpha and theta bands (unpublished data). These changes are present without significant motor manifestations, indicating that EEG changes in TAA-induced encephalopathy in rats appear before behavioral changes (Mladenović et al., 2012b). According to this finding, EEG changes are more relevant in the evaluation of mild TAA-induced encephalopathy than behavioral manifestations. Low doses of TAA induce EEG changes that partly correspond with mild HE in humans. Some studies found a frontal predominance of the alpha rhythm (Sagales et al., 1990; Montagnese et al., 2007), while others



**Fig. 1.** The effects of TAA on absolute (A) and relative power (B) in the **delta** band.

A daily dose of TAA (300 mg/kg) was administered once (TAA<sub>300</sub>), twice (TAA<sub>600</sub>) or three times (TAA<sub>900</sub>) on subsequent days. EEG was recorded 22.5–23.5 h after the administration of the last dose of TAA. The densities of absolute and relative power spectra were calculated with software using the fast Fourier transformation method. Significance of the differences in power spectra densities were estimated by two-way analysis of variance (ANOVA) with Tukey's post hoc test (\* $p < 0.05$  and \*\* $p < 0.01$  vs. control).



**Fig. 2.** The effects of TAA on absolute (A) and relative power (B) in the **theta** band. Significance of the differences in power spectra densities were estimated by two-way analysis of variance (ANOVA) with Tukey's post hoc test (\*\* $p < 0.01$  vs. control). For additional information see Fig. 1.

have described an increase in beta waves in mild human HE (Kullmann et al., 2001; Amodio et al., 2006). On the other hand, low-power beta activity was also described in the initial stages of HE in humans (Kullmann et al., 2001).

With an increasing dose of TAA, an initial decline in general motor activity and exploratory behavior is associated with an increase in absolute delta power spectra. This is the first EEG sign of bad prognosis in TAA-induced encephalopathy, since motor impairment was found to progress to a comatose state in some animals (Mladenović et al., 2012b; unpublished data). Further increase in dose of TAA induced an increase in relative delta power with a simultaneous decrease in the relative theta power (Figs. 1 and 2). This finding, in combination with the lower voltage of EEG waves, implies the worst outcome of TAA-induced encephalopathy, since coma usually developed in animals with these alterations (unpublished data). This is in accordance with clinical practice, since severe HE in humans is associated with a progressive slowing of the EEG (Guerit et al., 2009). The terminal stage of HE, hepatic coma, is accompanied by various EEG abnormalities in humans, from alpha rhythm admixed with theta slowing in early stages, to theta-delta slowing over both hemispheres in the late phases of coma. Triphasic waves may be present, but are not always evident (Amodio and Gatta, 2005). The late stage of coma is associated with a flattening of the EEG followed by a predominance of delta waves (Hunter and Young, 2010). Additionally, anteriorization and dissociation of the basic posterior alpha rhythm in combination with dissociation of frontal delta activities were observed with the progression of HE (Olesen et al., 2011).

For a more precise interpretation of EEG spectral analysis in human HE, Van der Rijt et al. (1984) introduced a classification scheme that can objectively assess the stage of HE. This classification is based on the mean dominant frequency and theta and delta relative power, and stages of HE are expressed by relative scores. According to this classification, the most severe form of HE corresponds to a mean dominant frequency of  $\leq 7.3$  Hz and delta relative power of  $\geq$

45% (Van der Rijt et al., 1984; Amodio et al., 1999a; 1999b). Although classification of EEG changes according to Van der Rijt et al. may be useful in humans (Amodio et al., 2006), it cannot be used for the evaluation of TAA-induced EEG changes in rats, since all TAA-treated groups in our study had a delta relative power of  $\geq 45\%$  (unpublished data). Furthermore, the mean score of EEG alterations did not differ between the TAA-treated and control group, making this scoring system inappropriate for TAA-induced encephalopathy (Mladenović et al., 2012b). However, the delta relative power remains an important parameter in the prognosis of TAA-induced encephalopathy (Fig. 1).

Recently, the artificial neural network-expert system procedure was introduced in the classification of HE in humans (Amodio et al., 2006). The possibility of implementing this classification in TAA-induced encephalopathy should be further evaluated.

#### *Mechanisms involved in the pathogenesis of TAA-induced encephalopathy*

TAA may induce encephalopathy by both direct and indirect mechanisms. Indirect mechanisms appear to be more important and are mediated primarily by liver failure-induced hyperammonemia (Reddy et al., 2004). Acute hyperammonemia induces astrocyte swelling, ultimately leading to cytotoxic brain edema and an increase in intracranial pressure (Norenberg, 1977; Jalan et al., 2003). However, not all studies have consistently shown brain edema after acute TAA treatment. The first study that described pathological changes in the brain in TAA-induced fulminant liver failure in rats accentuated neuronal degeneration in the CA<sub>1</sub> and CA<sub>4</sub> regions of the hippocampus (Peeling et al., 1993). Later, Norton et al., (1997) observed the shrinkage of pyramidal cells in CA<sub>1</sub> and CA<sub>2</sub> regions of hippocampus with vacuolization, slight cytoplasmic eosinophilia and pyknotic nuclei. Male rats displayed more pronounced pathological changes in stages III and IV of encephalopathy than female rats. In addition, no gross change in the morphology of the brain after TAA administration in Sprague-Dawley rats was found. No differences in the relative

weight of the brains were evident in the various stages of TAA-induced encephalopathy or between sexes (Norton et al., 1997). However, other studies have confirmed that TAA induces brain edema, similar to other causes of HE (Rama-Rao et al., 2010).

Astrocyte swelling in HE/hyperammonemia is attributed to the toxic effects of the glutamine (Gln) that is accumulated in the astrocytic mitochondria (Willard-Mack et al., 1996; Tanigami et al., 2005). The first hypothesis claimed that Gln, acting as an osmolyte, causes an increase in osmotic pressure in astrocytes with subsequent swelling (Brusilow and Traystman, 1986; Jalan et al., 2003). However, this hypothesis is difficult to accept, since Gln accounts for the minority of osmolytes in the brain (Pasantés-Morales et al., 2002) and *in vitro* studies confirmed a time delay between Gln formation and astrocyte swelling (Sinke et al., 2008). Nowadays, it is accepted that Gln causes brain edema by its accumulation in mitochondria, where it is degraded by phosphate-activated glutaminase into glutamate and ammonia. Newly released ammonia induces mitochondrial dysfunction with increased reactive oxygen species formation and mitochondrial permeability transition (the Trojan Horse hypothesis) (Albrecht et al., 2010). The tricarboxylic acid cycle and the malate-aspartate shuttle mechanism are the primary targets of ammonia in mitochondria (Faff-Michalak et al., 1991; Hertz et al., 2000; Hertz and Kala, 2007). The role of Gln as the Trojan Horse in astrocyte swelling was confirmed by the treatment of cultured astrocytes with histidine, the blocker of Gln transporter into mitochondria. Histidine completely blocks the overexpression of astrocytic aquaporin-4 in TAA-induced acute liver failure, as well as ammonia-induced activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein kinases (MAPK)-factors whose activation contribute to brain edema in hyperammonemia (Jayakumar et al., 2006; Sinke et al., 2008). This amino acid also prevented brain edema formation in TAA-treated rats (Rama-Rao et al., 2010), thus confirming that mitochondrial Gln has an important role in TAA-induced brain edema, similar to other causes of HE/hyperammonemia.

Hyperammonemia is also known to induce encephalopathy by changes in neurotransmission. Changes in neurotransmission in one brain area in HE may result in an alteration in other areas that are connected by neuronal circuits, thus contributing to an imbalance between various neurotransmitters (Cauli et al., 2009). The most described alterations are in glutamatergic and GABAergic transmission (Ahboucha and Butterworth, 2008; Felipo, 2008; Cauli et al., 2009; Miranda et al., 2010). Changes in glutamatergic transmission are responsible for acute hyperammonemia-induced death, as well as for the impairment of learning ability and motor function in chronic HE (Cauli et al., 2009). Acute hyperammonemia was found to induce neuronal death by excessive stimulation of glutamatergic N-methyl-D-aspartate (NMDA) receptors (excitotoxicity) (Hermenegildo et al., 2000). Although NMDA receptors may be involved in seizure appearance (Mladenović et al., 2007; Hrnčić et al., 2009), excessive stimulation of these receptors leads to excessive calcium influx into neurons (Kosenko et al., 2000) that subsequently leads to mitochondrial dysfunction, adenosine triphosphate (ATP) depletion (Kosenko et al., 1994), increased reactive oxygen species (ROS) production (Kosenko et al., 1999) and activation of calcium-dependent enzymes that cause neuronal damage (Cauli et al., 2007). The role of glutamate in neuronal damage in HE was confirmed by the protective effect of NMDA receptor antagonists in mice and rats with hyperammonemia (Hermenegildo et al., 1996; Vogels et al., 1997). Glutamate release was also found to be increased in the cortex of TAA-treated mice (Miranda et al., 2010), as well as in the hippocampus of rats (Moroni et al., 1983). Additionally, TAA inhibits the expression of the GLT-1 gene and protein, a glutamate transporter on astrocytes that contributes to the accumulation of glutamate in synaptic clefts (Norenberg et al., 1997a). These effects provide an additional support to the important role of hyperammonemia in the pathogenesis of TAA-induced encephalopathy.

Glutamatergic transmission is also altered in chronic HE. The glutamate-nitric oxide-cyclic guano-

sine monophosphate (cGMP) pathway is impaired in various animal models of chronic hyperammonemia and HE (Monfort et al., 2001; Rodrigo et al., 2007). Chronic hyperammonemia leads to the activation of calcium-calmodulin-protein kinase II (CaMKII) that phosphorylates neuronal nitric oxide synthase (nNOS) in Ser847, thus reducing the activity of this enzyme. In addition, chronic hyperammonemia is associated with the reduction of nNOS in synaptic membranes (ElMlili et al., 2010). These alterations in the glutamate-nitric oxide-cGMP pathway are responsible for learning and memory impairment in chronic HE (Cauli et al., 2009) and a similar mechanism may be relevant in the learning deficits in liver cirrhosis induced by TAA.

TAA injection also modulates GABAergic transmission (Ahboucha and Butterworth, 2008; Cauli et al., 2009). The levels of neurosteroids, allopregnanolone and tetrahydrodeoxycorticosterone are elevated in the brain tissue of TAA-treated mice (Itzhak et al., 1995), as well as in the brain of ammonia-injected mice (Norenberg et al., 1997b) and patients that have died in hepatic coma (Ahboucha et al., 2006). These neurosteroids are positive allosteric modulators of GABA<sub>A</sub> receptors, thus potentiating inhibitory neurotransmission in the brain in HE (Ahboucha and Butterworth, 2008). Some neurosteroids (pregnenolone) were found to reduce GABA<sub>A</sub> receptor activity and its level is also increased in HE (Cauli et al., 2009). The net effect of these counterregulating neurosteroids on the pathogenesis of HE, including the course of TAA-induced encephalopathy, should be further investigated.

Hyperammonemia-induced oxidative stress has also been found to contribute to the development of TAA-induced encephalopathy. NMDA receptors were found to participate in increased ROS production in the brain in acute hyperammonemia (Kosenko et al., 1999; Cauli et al., 2009). In addition, ROS production in TAA-induced HE may be increased as a result of mitochondrial dysfunction due to ammonia accumulation in this organelle (Albrecht et al., 2010). Oxidative stress contributes to astrocyte edema in acute HE

through the activation of MAPKs (Moriyama et al., 2010). Mladenović et al., (2012a) found that TAA induces lipid peroxidation in the brain in a dose-dependent manner with a simultaneous decrease in catalase activity. The role of oxidative stress in the neurotoxic effects of TAA was further confirmed by the reduction of manifestations of TAA-induced encephalopathy by antioxidants, such as vitamin E, L-carnitine and especially melatonin (Túnez et al., 2007). Dimethyl sulfoxide was also found to prevent TAA-induced oxidative brain injury and to improve the outcome of liver failure (Túnez et al., 2005). However, all brain regions are not equally sensitive to oxidative stress. According to our (Mladenović et al., 2012a) and other data (Sathyaikumar et al., 2007), the hippocampus and cerebellum were found to be less sensitive to lipid peroxidation than the cerebral cortex and brainstem, while the brainstem showed the greatest susceptibility among these structures.

Mechanisms that may be responsible for the different effects of TAA on various brain regions may include the different metabolic rate of TAA in various brain regions, the different expression of antioxidant enzymes in brain regions, differences in the iron content in various parts of the brain or the different expression of the genes involved in oxidative metabolism, deoxyribonucleic acid (DNA) repair, ribonucleic acid (RNA) processing and genes involved in various signaling pathways (Wang et al., 2009). One of the possible mechanisms of different vulnerability of brain regions to TAA-induced lipid peroxidation may be, according to our study (Mladenović et al., 2012a), the different effects of TAA on catalase activity in the cortex, brainstem and hippocampus. In addition, increased NO levels, increased activity of superoxide dismutase with decreased glutathione peroxidase and glutathione reductase activities may also be responsible for the highest susceptibility of the brainstem to TAA-induced oxidative stress (Sathyaikumar et al., 2007).

Apart from lipid peroxidation and oxidative stress, TAA was found to induce changes in lipid contents and the fluidity of cell membranes in the

cortex (Swapna et al., 2006). The precise relation between membrane changes and lipid peroxidation remains controversial. TAA causes a decrease in the unsaturated to saturated fatty acid ratio, as well as a decrease in cholesterol, sphingomyelin and phosphatidylserine level in cerebral membranes (Swapna et al., 2006). Decreased levels of cholesterol and sphingomyelin in the membranes influence lipid raft formation, receptor and neurotransmitter functions, as well as glutamate uptake by the cortical neurons (Felipo and Butterworth, 2002). In accordance with these findings, cholesterol depletion was found to increase the vulnerability of neurons to glutamate excitotoxicity (Chou et al., 2003). Excitotoxicity contributes to ROS formation in neurons (Kosenko et al., 1999), thus indicating that changes in membrane lipid composition may contribute to increased lipid peroxidation in the cortex after TAA administration. On the other hand, lipid peroxidation induces an increase in membrane fluidity, also evident in models of TAA-induced HE (Ghosh et al., 1993; Swapna et al., 2006).

Although hyperammonemia has a central role in the neurotoxic effects of TAA, the direct toxicity of this hepatotoxin on the brain cannot be excluded. It is known that TAA is oxidized in the liver to thioacetamide-S,S-dioxide by a cytochrome P450 isoenzyme, CYP2E1 (Wang et al., 2001). CYP2E1 is also present in various brain regions and this enzyme may contribute to the generation of toxic TAA metabolites in the brain, which may be responsible for the direct neurotoxic effects of TAA (Wu et al., 2011). These metabolites are evident in the brain after a 3-day intraperitoneal treatment of rats with TAA (Peeling et al., 1993). On the other hand, one study on TAA-induced cirrhosis excludes the possibility of the contribution of direct neurotoxic effects of TAA to the development of encephalopathy in that model (Méndez et al., 2008a). According to this study, TAA was metabolized during the first pass through the liver, since it was administered in drinking water. However, levels of TAA metabolites were not measured in this study, so the contribution of direct neurotoxic effects to the development of encephalopathy should be further evaluated.

## CONCLUSION

Although there are no ideal experimental models of HE, TAA is widely used for the induction of acute or chronic liver failure (Norton et al., 1997; Méndez et al., 2008a; 2008b; Miranda et al., 2010; Mladenović et al., 2012a; 2012b). TAA induces behavioral and EEG changes that correlate with human HE in a dose-dependent manner (Mladenović et al., 2012b). The first manifestations of TAA-induced encephalopathy are an increase in relative alpha power and a decrease in relative delta power, with a simultaneous increase in voltage in beta, alpha and theta bands. These EEG manifestations are not accompanied by motor changes. An increase in TAA dose induces a decline in general motor activity, associated with an increase in delta wave voltage. With further increases in dose, behavioral manifestations progress to coma with a flattening of the EEG with dominant activity in the delta band (Mladenović et al., 2012b, unpublished data). These manifestations may be caused by the direct and indirect mechanisms of TAA on the brain. Indirect mechanisms appear to be more important and are mediated by liver failure-induced hyperammonemia (Reddy et al., 2004). Hyperammonemia causes astrocyte swelling (Norenberg, 1977; Jalan et al., 2003), alterations in glutamatergic and GABAergic neurotransmission (Hermenegildo et al., 2000; Cauli et al., 2009; Ahboucha and Butterworth, 2008), oxidative stress (Kosenko et al., 1999), calcium accumulation in neurons and mitochondrial dysfunction with subsequent neuronal damage (Kosenko et al., 2000; Cauli et al., 2007).

The mechanisms of the direct neurotoxicity of TAA are not completely understood, but appear to be associated with the formation of toxic metabolites in the brain (Wang et al., 2001). However, TAA-induced encephalopathy remains a reliable model for the further investigation of the pathogenesis in HE and potential therapeutic procedures.

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## REFERENCES

- Aguilar, M.A., Miñarro, J., and V. Felipo (2000). Chronic moderate hyperammonemia impairs active and passive avoidance behaviour and conditional discrimination learning in rats. *Exp. Neurol.* **161**, 704-713.
- Ahboucha, S., and R.F. Butterworth (2008). The neurosteroid system: implication in the pathophysiology of hepatic encephalopathy. *Neurochem. Inter.* **52**, 575-587.
- Ahboucha, S., Pomier-Layrargues, G., Mamer, O., and R.F. Butterworth (2006). Increased levels of pregnenolone and its neuroactive metabolite allopregnanolone in autopsied brain tissue from cirrhotic patients who died in hepatic coma. *Neurochem. Int.* **49**, 372-378.
- Albrecht, J., Hilgier, W., and U. Rafalowska (1990). Activation of arginine metabolism to glutamate in rat brain synaptosomes in thioacetamide-induced hepatic encephalopathy: An adaptive response? *J. Neurosci. Res.* **25**, 125-130.
- Albrecht, J., and E.A. Jones (1999). Hepatic encephalopathy: molecular mechanisms underlying the clinical syndrome. *J. Neurol. Sci.* **170**, 138-146.
- Albrecht, J., Zielińska, M., and M.D. Norenberg (2010). Glutamine as a mediator of ammonia neurotoxicity: A critical appraisal. *Biochem. Pharmacol.* **80**, 1303-1308.
- Amodio, P., Del Piccolo, F., Marchetti, P., Angeli, P., Iemmolo, R., Caregaro, L., Merkel, C., Gerunda, G., and A. Gatta (1999a). Clinical features and survival of cirrhotic patients with subclinical cognitive alterations detected by the number connection test and computerized psychometric tests. *Hepatology* **29**, 1662-1667.
- Amodio, P., and A. Gatta (2005). Neurophysiological investigation of hepatic encephalopathy. *Metab. Brain Dis.* **20**, 369-739.
- Amodio, P., Marchetti, P., Del Piccolo, F., de Tourtchaninoff, M., Varghese, P., Zuliani, C., Campo, G., Gatta, A., and J.M. Guérit (1999b). Spectral versus visual EEG analysis in mild hepatic encephalopathy. *Clin. Neurophysiol.* **110**, 1334-1344.
- Amodio, P., Pellegrini, A., Ubiali, E., Mathy, I., Piccolo, F.D., Orsato, R., Gatta, A., and J.M. Guérit (2006). The EEG assessment of low-grade hepatic encephalopathy: Comparison of an artificial neural network-expert system (ANNES) based evaluation with visual EEG readings and EEG spectral analysis. *Clin. Neurophysiol.* **117**, 2243-2251.
- Avraham, Y., Israeli, E., Gabbay, E., Okun, A., Zolotarev, O., Silberman, I., Ganzburg, V., Dagon, Y., Magen, I., Vorobia, L., Pappo, O., Mechoulam, R., Ilan, Y., and E.M. Berry (2006). Endocannabinoids affect neurological and cognitive function in thioacetamide-induced hepatic encephalopathy in mice. *Neurobiol. Dis.* **21**, 237-245.
- Brusilow, S.W., and R. Traystman (1986). Letter to the editor. *N. Engl. J. Med.* **314**, 786-797.
- Cauli, O., López-Larrubia, P., Rodrigues, T.B., Cerdán, S., and V. Felipo (2007). Magnetic resonance analysis of the effects of acute ammonia intoxication on rat brain. Role of NMDA receptors. *J. Neurochem.* **103**, 1334-1343.
- Cauli, O., Rodrigo, R., Llansola, M., Montoliu, C., Monfort, P., Piedrafitá, B., El Mlili, N., Boix, J., Agusti, A., and V. Felipo (2009). Glutamatergic and GABAergic neurotransmission and neuronal circuits in hepatic encephalopathy. *Metab. Brain Dis.* **24**, 69-80.
- Chou, Y.C., Lin, S.B., Tsai, L.S., Tsai, H.I., and C.M. Lin (2003). Cholesterol deficiency increases the vulnerability of hippocampal glia in primary culture to glutamate-induced excitotoxicity. *Neurochem. Int.* **43**, 197-209.
- Córdoba, J. (2011). New assessment of hepatic encephalopathy. *J. Hepatol.* **54**, 1030-1040.
- Díez-Fernández, C., Sanz, N., and M. Cascales (1996). Intracellular calcium concentration impairment in hepatocytes from thioacetamide-treated rats. Implications for the activity of Ca<sup>2+</sup>-dependent enzymes. *J. Hepatol.* **24**, 460-467.
- Dyroff, M.C., and R.A. Neal (1981). Identification of the major protein adduct formed in rat liver after thioacetamide administration. *Cancer Res.* **41**, 3430-3435.
- ElMlili, N., Boix, J., Ahabrach, H., Rodrigo, R., Errami, M., and V. Felipo (2010). Chronic hyperammonemia induces tonic activation of NMDA receptors in cerebellum. *J. Neurochem.* **112**, 1005-1014.
- Faff-Michalak, L., and J. Albrecht (1991). Aspartate aminotransferase, malate dehydrogenase, and pyruvate carboxylase activities in rat cerebral synaptic and non-synaptic mitochondria: effects of in vitro treatment with ammonia, hyperammonemia and hepatic encephalopathy. *Metab. Brain Dis.* **6**, 187-197.
- Felipo, V. (2008). Hyperammonemia. *Handbook of neurochemistry and molecular neurobiology*. Vol. 24, 3<sup>rd</sup> ed. pp. 1-27, Kluwer Academic/Plenum, USA.
- Felipo, V., and R. Butterworth (2002). Neurobiology of ammonia. *Prog. Neurobiol.* **67**, 259-279.
- Gammal, S.H., and E.A. Jones (1989). Hepatic encephalopathy. *Med. Clin. North Am.* **73**, 793-813.
- Ghosh, C., Dick, R.M., and S.F. Ali (1993). Iron/ascorbate-induced lipid peroxidation changes membrane fluidity and muscarinic cholinergic receptor binding in rat frontal cortex. *Neurochem. Int.* **23**, 479-484.

- Guerit, J.M., Amantini, A., Fischer, C., Kaplan, P.W., Mecarelli, O., Schnitzler, A., Ubiali, E., Amodio, P. and the members of the ISHEN commission on Neurophysiological Investigations. (2009). Neurophysiological investigations of hepatic encephalopathy: ISHEN practice guidelines. *Liver Inter.* **29**, 789-796.
- Hagve, T.A., Christensen, E., Gronn, M., and B.O. Christophersen (1988). Regulation of the metabolism of polyunsaturated fatty acids. *Scand. J. Clin. Lab. Invest. Suppl.* **191**, 33-46.
- Hermenegildo, C., Marcaida, G., Montoliu, C., Grisolia, S., Miñana, M.D., and V. Felipo (1996). NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochem. Res.* **21**, 1237-1244.
- Hermenegildo, C., Monfort, P., and V. Felipo (2000). Activation of NMDA receptors in rat brain in vivo following acute ammonia intoxication. Characterization by in vivo brain microdialysis. *Hepatology* **31**, 709-715.
- Hertz, L., and Kala, G. (2007). Energy metabolism in brain cells: effects of elevated ammonia concentrations. *Metab. Brain Dis.* **22**, 199-218.
- Hertz, L., Yu, A.C., Kala, G., and A. Schousboe (2000). Neuronal-astrocytic and cytosolic-mitochondrial metabolite trafficking during brain activation, hyperammonemia and energy deprivation. *Neurochem. Int.* **37**, 83-102.
- Hilgier, W., Benveniste, H., Diemer, N.H., and J. Albrecht (1991). Decreased glucose utilization in discrete brain regions of rat in thioacetamide-induced hepatic encephalopathy as measured with [<sup>3</sup>H]-deoxyglucose. *Acta Neurol. Scand.* **83**, 353-355.
- Hrnčić, D., Rašić-Marković, A., Šušić, V., Djuric, D., and O. Stanojlović (2009). Influence of NR2B-selective NMDA antagonist on lindane-induced seizures in rats. *Pharmacology* **84**, 234-239.
- Hunter, A.L., Holscher, M.A., and R.A. Neal (1977). Thioacetamide-induced hepatic necrosis. I. Involvement of the mixed-function oxidase enzyme system. *J. Pharmacol. Exp. Ther.* **200**, 439-448.
- Hunter, G.R., and G.B. Young (2010). Recovery of awareness after hyperacute hepatic encephalopathy with "flat" EEG, severe brain edema and deep coma. *Neurocrit. Care* **13**, 247-251.
- Itzhak, Y., Roig-Cantisano, A., Dombro, R.S., and M.D. Norenberg (1995). Acute liver failure and hyperammonemia increase peripheral-type benzodiazepine receptor binding and pregnenolone synthesis in mouse brain. *Brain Res.* **705**, 345-348.
- Jalan, R., Shawcross, D., and N. Davies (2003). The molecular pathogenesis of hepatic encephalopathy. *Inter. J. Biochem. Cell. Biol.* **35**, 1175-1181.
- Jayakumar, A.R., Panickar, K.S., Murthy, Ch.R., and M.D. Norenberg (2006). Oxidative stress and mitogen-activated protein kinase phosphorylation mediate ammonia-induced cell swelling and glutamate uptake inhibition in cultured astrocytes. *J. Neurosci.* **26**, 4774-4784.
- Kamataki, T., Maeda, K., Shimada, M., and R. Kato (1986). Effects of phenobarbital, 3-methylcholanthrene and polychlorinated biphenyls on sex-specific forms of cytochrome P450 in liver microsomes of rats. *J. Biochem. Tokyo* **99**, 841-845.
- Kosenko, E., Kaminsky, Y., Grau, E., Miñana, M.D., Marcaida, G., Grisolia, S., and V. Felipo (1994). Brain ATP depletion induced by acute ammonia intoxication in rats is mediated by activation of the NMDA receptor and of Na/K ATP-ase. *J. Neurochem.* **63**, 2172-2178.
- Kosenko, E., Kaminsky, Y., Lopata, O., Muravyov, N., and V. Felipo (1999). Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication. *Free Rad. Biol. Med.* **26**, 1369-1374.
- Kosenko, E., Kaminsky, Y., Stavroskaya, I.G., and V. Felipo (2000). Alteration of mitochondrial calcium homeostasis by ammonia-induced activation of NMDA receptors in rat brain in vivo. *Brain Res.* **880**, 139-146.
- Kullmann, F., Hollerbach, S., Lock, G., Holstege, A., Dierks, T., and J. Schölmerich (2001). Brain electrical activity mapping of EEG for the diagnosis of (sub)clinical hepatic encephalopathy in chronic liver disease. *Eur. J. Gastroenterol. Hepatol.* **13**, 513-522.
- Méndez, M., Méndez-López, M., Lopez, L., Aller, M.A., Arias, J., and J.L. Arias (2008a). Mammillary body alterations and spatial memory impairment in Wistar rats with thioacetamide-induced cirrhosis. *Brain Res.* **1233**, 185-195.
- Méndez, M., Méndez-López, M., Lopez, L., Aller, M.A., Arias, J., Cimadevilla, J.M., and J.L. Arias (2008b). Spatial memory alterations in three models of hepatic encephalopathy. *Behav. Brain Res.* **188**, 32-40.
- Miranda, A.S., Rodrigues, D.H., Vieira, L.B., Lima, C.X., Rachid, M.A., Vidigal, P.V., Gomez, M.V., Reis, H.J., Guatimosim, C., and A.L. Teixeira (2010). A thioacetamide-induced hepatic encephalopathy model in C57BL/6 mice. a behavioral and neurochemical study. *Arq. Neuropsiquiatr.* **68**, 597-602.
- Mladenović, D., Krstic, D., Colovic, M., Radosavljević, T., Rašić-Marković, A., Hrnčić, D., Macut, Dj., and O. Stanojlović (2012a). Different sensitivity of various brain structures to thioacetamide-induced lipid peroxidation. *Med. Chem.* **8**, 52-58.
- Mladenović, D., Hrnčić, D., Vučević, D., Radosavljević, T., Loncar-Stevanović, H., Petrović, J., Susic, V., Djuric, D., and O. Stanojlović (2007). Ethanol suppressed seizures in lin-

- dane- treated rats. Electroencephalographic and behavioral studies. *J. Physiol. Pharmacol.* **58**, 641-654.
- Mladenović, D., Radosavljević, T., Hrnčić, D., Rašić-Marković, A., Puškaš, N., Maksić, N., Djuric, D., and O. Stanojlović (2012b). Behavioral and electroencephalographic manifestations of thioacetamide-induced hepatic encephalopathy in rats. *Can. J. Physiol. Pharmacol.* (in press).
- Monfort, P., Corbalán, R., Martínez, L., López-Talavera, J.C., Córdoba, J., and V. Felipo (2001). Altered content and modulation of soluble guanylate cyclase in the cerebellum of rats with portacaval anastomosis. *Neuroscience* **104**, 1119-1125.
- Monfort, P., Muñoz, M.D., and V. Felipo (2004). Hyperammonemia impairs long-term potentiation in hippocampus by altering the modulation cAMP-degrading phosphodiesterase by protein kinase G. *Neurobiol. Dis.* **15**, 1-10.
- Montagnese, S., Jackson, C., and M.Y. Morgan (2007). Spatiotemporal decomposition of the electroencephalogram in patients with cirrhosis. *J. Hepatol.* **46**, 447-458.
- Mori, W., and H. Aoyama (1983). Statistical study on fulminant liver disease in Japan. *Appl. Pathol.* **1**, 209-222.
- Moriyama, M., Jayakumar, A.R., Tong, X.Y., and M.D. Norenberg (2010). Role of mitogen-activated protein kinases in the mechanism of oxidant-induced cell swelling in cultured astrocytes. *J. Neurosci. Res.* **88**, 2450-2458.
- Moroni, F., Lombardi, G., Moneti, G., and C. Cortesini (1983). The release and neosynthesis of glutamic acid are increased in experimental models of hepatic encephalopathy. *J. Neurochem.* **40**, 850-854.
- Muñoz, D., Monfort, P., and V. Felipo (2000). Hyperammonemia impairs NMDA receptor-dependent long-term potentiation in the CA1 of rat hippocampus in vitro. *Neurochem. Res.* **25**, 437-441.
- Norenberg, M.D. (1977). A light and electron microscopic study of experimental portal-systemic (ammonia) encephalopathy. Progression and reversal of the disorder. *Lab. Invest.* **36**, 618-627.
- Norenberg, M.D., Huo, Z., Neary, J.T., and A. Roig-Cantesano (1997a). The glial glutamate transporter in hyperammonemia and hepatic encephalopathy: relation to energy metabolism and glutamatergic neurotransmission. *Glia* **21**, 124-133.
- Norenberg, M.D., Itzhak, Y., and A.S. Bender (1997b). The peripheral benzodiazepine receptor and neurosteroids in HE. *Adv. Exp. Med. Biol.* **420**, 95-111.
- Norton, N.S., McConnell, J.R., and J.F. Rodriguez-Sierra (1997). Behavioral and physiological sex differences observed in an animal model of fulminant hepatic encephalopathy in the rat. *Physiol. Behav.* **62**, 1113-1124.
- Nozu, F., Takeyama, N., and T. Tanaka (1992). Changes of hepatic fatty acid metabolism produced by chronic thioacetamide administration in rats. *Hepatology* **15**, 1099-1106.
- Olesen, S.S., Graversen, C., Hansen, T.M., Blauenfeldt, R.A., Hansen, J.B., Steimle, K., and A.M. Drewes (2011). Spectral and dynamic electroencephalogram abnormalities are correlated to psychometric test performance in hepatic encephalopathy. *Scand. J. Gastroenterol.* **46**, 988-996.
- Pasantes-Morales, H., Franco, R., Ordaz, B., and L.D. Ochoa (2002). Mechanisms counteracting swelling in brain cells during hyponatremia. *Arch. Med. Res.* **33**, 237-244.
- Peeling, J., Shoemaker, L., Gauthier, T., Benarroch, A., Sutherland, G.R., and G.Y. Minuk (1993). Cerebral metabolic and histological effects of thioacetamide-induced liver failure. *Am. J. Physiol.* **265**, G572-G578.
- Porter, W.R., Gudzinowicz, M.J., and R.A. Neal (1979). Thioacetamide-induced hepatic necrosis: II. Pharmacokinetics of thioacetamide and thioacetamide-S-oxide in the rat. *J. Pharmacol. Exp. Ther.* **208**, 386-391.
- Rama-Rao, K.V., Reddy, P.V., Tong, X., and M.D. Norenberg (2010). Brain edema in acute liver failure: inhibition by L-histidine. *Am. J. Pathol.* **176**, 1400-1408.
- Reddy, P.V.B., Murthy, Ch.R.K., and P. Reddanna (2004). Fulminant hepatic failure induced oxidative stress in nonsynaptic mitochondria of cerebral cortex in rats. *Neurosci. Lett.* **368**, 15-20.
- Rodrigo, R., Erceg, S., and V. Felipo (2005). Neurons exposed to ammonia reproduce the differential alteration in nitric oxide modulation of guanylate cyclase in the cerebellum and cortex of patients with liver cirrhosis. *Neurobiol. Dis.* **19**, 150-161.
- Rodrigo, R., Erceg, S., Rodriguez-Diaz, J., Saez-Valero, J., Piedrafita, B., Suarez, I., and V. Felipo (2007). Glutamate-induced activation of nitric oxide synthase is impaired in cerebral cortex in vivo in rats with chronic liver failure. *J. Neurochem.* **102**, 51-64.
- Rogers, D.C., Fisher, E.M., Brown, S.D., Peters, J., Hunter, A.J., and J.E. Martin (1997). Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm. Genome* **8**, 711-713.
- Sagales, T., Gimeno, V., de la Calzada, M.D., Casellas, F., Dolores-Macià, M., and M. Villar-Soriano (1990). Brain mapping analysis in patients with hepatic encephalopathy. *Brain Topog.* **2**, 221-228.
- Sanz, N., Díez-Fernández, C., Fernández-Simón, L., Alvarez, A., and M. Cascales, (1998). Necrogenic and regenerative responses of liver of newly weaned rats against a sublethal dose of thioacetamide. *Biochim. Biophys. Acta* **1384**, 66-78.

- Sathyasaikumar, K.V., Swapna, I., Reddy, P.V., Murthy, Ch.R., Dutta Gupta, A., Senthilkumaran, B., and P. Reddanna (2007). Fulminant hepatic failure in rats induces oxidative stress differentially in cerebral cortex, cerebellum and pons medulla. *Neurochem. Res.* **32**, 517-524.
- Sawant, S.P., Dnyanmote, A.V., Warbritton, A., Latendresse, J.R., and H.M. Mehendale (2006). Type 2 diabetic rats are sensitive to thioacetamide hepatotoxicity. *Toxicol. Appl. Pharmacol.* **211**, 221-232.
- Sinke, A.P., Jayakumar, A.R., Panickar, K.S., Moriyama, M., Reddy, P.V., and M.D. Norenberg (2008). NFkappaB in the mechanism of ammonia-induced astrocyte swelling in culture. *J. Neurochem.* **106**, 2301-2311.
- Swapna, I., Sathyasaikumar, K.V., Murthy, Ch.R.K., Dutta-Gupta, A., and B. Senthilkumaran (2006). Changes in cerebral membrane lipid composition and fluidity during thioacetamide-induced hepatic encephalopathy. *J. Neurochem.* **98**, 1899-1907.
- Tanigami, H., Rebel, A., Martin, L.J., Chen, T.Y., Brusilow, S.W., Traystman, R.J., and R.C. Koehler (2005). Effect of glutamine synthetase inhibition on astrocyte swelling and altered astroglial protein expression during hyperammonemia in rats. *Neuroscience* **131**, 437-449.
- Terblanche, J., and R. Hickman (1991). Animal models of fulminant hepatic failure. *Dig. Dis. Sci.* **36**, 770-774.
- Túnez, I., Muñoz, M.C., Medina, F.J., Salcedo, M., Feijóo, M., and P. Montilla (2007). Comparison of melatonin, vitamin E and L-carnitine in the treatment of neuro- and hepatotoxicity induced by thioacetamide. *Cell. Biochem. Funct.* **25**, 119-127.
- Túnez, I., Muñoz, M.C., Villavicencio, M.A., Medina, F.J., de Prado, E.P., Espejo, I., Barcos, M., Salcedo, M., Feijóo, M., and P. Montilla (2005). Hepato- and neurotoxicity induced by thioacetamide: protective effects of melatonin and dimethyl sulfoxide. *Pharmacol. Res.* **52**, 223-228.
- Uehara, T., Hirode, M., Ono, A., Kiyosawa, N., Omura, K., Shimizu, T., Mizukawa, Y., Miyagishima, T., and T. Nagao (2008). A toxicogenomics approach for early assessment of potential non-genotoxic hepatocarcinogenicity of chemicals in rats. *Toxicology* **250**, 15-26.
- Van der Rijt, C.C., Schalm, S.W., De Groot, G.H., and M. De Vlioger (1984). Objective measurement of hepatic encephalopathy by means of automated EEG analysis. *Electroencephalogr. Clin. Neurophysiol.* **57**, 423-426.
- Vogels, B.A., Maas, M.A., Daalhuisen, J., Quack, G., and R.A. Chamuleau (1997). Memantine, a noncompetitive NMDA receptor antagonist improves hyperammonemia-induced encephalopathy and acute hepatic encephalopathy in rats. *Hepatology* **25**, 820-827.
- Wang, T., Shankar, K., Bucci, T.J., Warbritton, A., and H.M. Mehendale (2001). Diallyl sulfide inhibition of CYP2E1 does not rescue diabetic rats from thioacetamide-induced mortality. *Toxicol. Appl. Pharmacol.* **173**, 27-37.
- Wang, X., Zaidi, A., Pal, R., Garrett, A.S., Bracerias, R., Chen, X.W., Michaelis, M.L., and E.K. Michaelis (2009). Genomic and biochemical approaches in the discovery of mechanisms for selective neuronal vulnerability to oxidative stress. *BMC Neurosci.* **10**, 12.
- Wesierska, M., Klinowska, H.D., Adamska, I., Fresko, I., Sadowska, J., and J. Albrecht (2006). Cognitive flexibility but not cognitive coordination is affected in rats with toxic liver failure. *Behav. Brain Res.* **171**, 70-77.
- Willard-Mack, C.L., Koehler, R.C., Hirata, T., Cork, L.C., Takahashi, H., Traystman, R.J., and S.W. Brusilow (1996). Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. *Neuroscience* **71**, 589-599.
- Wu, J.Y., Yue, J., and Y.Q. Feng (2011). Determination of brain cytochrome P450 2E1 activity in rat with the probe of chlorzoxazone by liquid chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **879**, 260-266.
- Yogalakshmi, B., Viswanathan, P., and C.V. Anuradha (2010). Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. *Toxicology* **268**, 204-212.

