Chapter 15
The Effects of Chronic Taurine Supplementation on Motor Learning

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Abstract Taurine is one of the most abundant nonprotein amino acids shown to be essential for the development, survival, and growth of vertebrate neurons. We previously demonstrated that chronic taurine supplementation during neonatal development results in changes in the GABAergic system (El Idrissi, Neurosci Lett 436:19–22, 2008). The brains of mice chronically treated with taurine have decreased levels of $GABA_A$ β subunits and increased expression of GAD and GABA, which contributes to hyperexcitability. This down regulation of $GABA_A$ receptor subunit expression and function may be due to a sustained interaction of taurine with $GABA_A$ receptors. This desensitization decreases the efficacy of the inhibitory synapses at the postsynaptic membrane. If changes occur in the GABAergic system as a possible compensatory mechanism due to taurine administration, then it is important to study all aspects by which taurine induces hyperexcitability and affects motor behavior. We therefore hypothesized that modification of the GABAergic system in response to taurine supplementation influences motor learning capacity in mice. To test this hypothesis, the rotarod task was employed after chronic taurine supplementation in drinking water (0.05% for 4 weeks). Control animals receiving no taurine supplementation were also tested in order to determine the difference in motor learning ability between groups. Each animal was trained on the rotarod apparatus for 7 days at an intermediate speed of 24 rpm in order to establish baseline performance. On the testing day, each animal was subjected to eight different predefined speeds (5, 8, 15, 20, 24, 31, 33, and 44 rpm). From our observations, the animals

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that underwent chronic taurine supplementation appeared to have a diminished motor learning capacity in comparison to control animals. The taurine-fed mice displayed minor improvements after repeated training when compared to controls. During the testing session the taurine-fed mice also exhibited a shorter latency to fall, as the task requirements became more demanding.

15.1 Introduction

Taurine, 2-aminoethanesulfonic acid, is the second most abundant nonprotein amino acid in the central nervous system of mammals (Huxtable and Lleu 1992). Taurine is crucial for the development, survival, and growth of vertebrate neurons (Hayes et al. 1975). High concentrations of taurine are incorporated into fetal and early postnatal rodents via their mothers (Sturman et al. 1977). Within the brain, taurine concentrations increase until weaning, and subsequently decline reaching stable concentrations in adulthood that are comparable, but second to those of glutamate which is the main excitatory neurotransmitter. Activation of glutamate receptors leads to a depolarization of the postsynaptic membrane causing extracellular calcium influx as well as mobilization of calcium from intracellular stores (Jaffe and Brown 1994). Many physiological processes rely on calcium as a vital second messenger (Kater et al. 1988), but despite that, excessive elevation of intracellular calcium levels results in structural damage to neurons (El Idrissi and Trenkner 2004). Hyperexcitability of the brain is prevented by γ-aminobutyric acid (GABA), the predominant inhibitory neurotransmitter. When GABA is released from presynaptic neurons, it acts by binding to the ionotropic $GABA_A$ receptor located on the postsynaptic neuron. The outcome of this activity permits chloride influx, and subsequent hyperpolarization of the postsynaptic membrane.

Taurine is structurally related to GABA and acts itself as an inhibitory amino acid during development. Taurine is a partial agonist of the $GABA_A$ receptor (Frosini et al. 2003), and activates chloride influx into postsynaptic neurons through this receptor (El Idrissi and Trenkner 2004). Increases in chloride concentrations within the cell results in hyperpolarization of the postsynaptic membrane, and therefore reduces excitability. In addition to acting as a partial agonist of the $GABA_A$ receptor, taurine has also been shown to activate the corticostriatal pathway by behaving as an endogenous ligand for glycine receptors (Chepkova et al. 2002). Moreover, taurine has been shown to activate Cl^- influx through $GABA_A$ receptors in cerebellar granule cells in vitro (El Idrissi and Trenkner 2004). In the same study, cultures were pretreated with taurine (1 mM) for 24 h prior to the addition of glutamate, and afterwards Ca^{2+} uptake was shown to be significantly lower than in control cultures. Taurine also prevents neuronal damage associated with excitotoxicity. This is achieved through the regulation of cytoplasmic and intramitochondrial calcium homeostasis after glutamate receptor activation (El Idrissi and Trenkner 1999). The mechanisms by which taurine accomplishes modulation of neuronal excitability are direct enhancement of GABAergic function and indirect

depression of glutamatergic neurotransmission (El Idrissi and Trenkner 2004). During development of the nervous system, when GABA is excitatory, taurine might play a critical role as a regulator of neuronal excitability through calcium modulation, and thereby, compensate for the lack of receptor-mediated neuronal inhibition (El Idrissi and Trenkner 2004). Furthermore, taurine may also play a vital role in neuroprotection since levels in the brain significantly increase during stressful conditions (Wu et al. 1998).

Taurine deficiency has been established in a number of neuropathological disorders, such as epilepsy (Barbeau et al. 1975; Joseph and Emson 1976), mental depression (Perry 1976), and alcohol withdrawal syndrome (Ikeda 1977). To date, taurine supplementation has been demonstrated to affect behavior in rodents. For example, acute taurine injections have been shown to increase the threshold of pharmacologically induced convulsions, and also significantly improved survivability when compared to controls (El Idrissi et al. 2003). On the other hand, chronic supplementation of taurine in drinking water increases brain excitability in mice, which occurs mainly through alteration in the inhibitory GABAergic system (L'Amoreaux et al. 2010). Chronic taurine supplementation induces down regulation of $GABA_A$ receptor expression due to a sustained interaction of taurine with $GABA_A$ receptors. This process decreases the efficacy of the inhibitory synapses at the postsynaptic membrane. The β subunit of the $GABA_A$ receptor is a key subunit that is present in virtually all of these receptors (Barnard et al. 1998) and is considered to be required for receptor assembly and function (Connolly et al. 1996). Within the hippocampus of taurine-fed mice, a reduction of $GABA_A$ β subunit expression was observed (El Idrissi 2006). In conjunction with this, the taurine-fed mice had reduced expression of $GABA_A$ receptors in the hippocampus.

Motor coordination is an acquired skill that manifests through a process of adaptation. Learning to walk, swim, ride a bike, or excel at a physical sport are examples of motor learning (Crawley 2007). The learning of skilled movement is controlled by interactions between the supplementary motor area, prefrontal parietal cortex, basal ganglia, and cerebellum (Rustay et al. 2003). The basal ganglia are involved with the automatic execution of learned motor plans, and in the preparation of movement (Afifi 2003). When cortical signals are received and processed by basal ganglia systems, the suppression of competing motor programs occurs when the neurotransmitter GABA inhibits thalamic nuclei. Thalamic nuclei provide the link between the basal ganglia and the motor, supplementary motor, premotor, prefrontal, and limbic cortices (Afifi 2003). On the other hand, the cerebellum influences movements primarily by modifying the activity patterns of the upper motor neurons located in the cerebral cortex. The primary function of the cerebellum is evidently to detect the difference, or "motor error," between an intended movement and the actual movement, and, through its influence over upper motor neurons, to reduce the error (Purves et al. 2008). Therefore, the cerebellum is the fundamental processing center required for the learning of compound movements. Furthermore, the cerebellar circuitry is highly reliant on synaptic integration derived from GABA-mediated inhibition.

GABAergic modification plays an important part in motor learning and plasticity. If changes occur in the GABAergic system as a possible compensatory mechanism due to taurine administration, then it is important to study all aspects by which chronic taurine supplementation induces hyperexcitability, and affects behavior. We therefore hypothesized that modification of the GABAergic system in response to taurine supplementation influences motor learning capacity in mice. To test this hypothesis, the rotarod task was employed after chronic taurine supplementation. Control animals receiving no taurine supplementation were also tested in order to determine the difference in motor learning ability between groups. From our observations, the animals that underwent chronic taurine supplementation appeared to have a diminished motor learning capacity in comparison to control animals.

15.2 Methods

15.2.1 Rotarod Task

Experiments were carried out on FVB/NJ adult male mice. Animals were given either distilled water and served as controls or a solution of taurine dissolved in distilled water at 0.05%. After 4 weeks of chronic taurine supplementation rotarod task was employed. The rotarod (IITC Life Science Inc., Woodland Hills, CA) is an apparatus which is used to gauge the ability of an animal to maintain balance on a rotating rod as well as motor learning ability in rodents (Lalonde et al. 1995). Three measures were obtained during each trial from the apparatus: (1) latency to fall measured in seconds, (2) distance traveled prior to fall measured in meters, and (3) animal velocity.

During the training phase, the rotation was set to an intermediate speed (24 rpm) in order to establish baseline performance. Each mouse was placed on the rotating rod for a maximum of 60 s. Latency to fall off the rotarod was recorded within this time period. Each animal underwent five trials per day for 7 days. After each trial, the animal was returned to its home cage for an intertrial interval (ITI) of 5 min. On the testing day, each animal was subjected to eight different predefined speeds (5, 8, 15, 20, 24, 31, 33, and 44 rpm). The mice were given two trials at each speed level with an ITI of 5 min.

15.2.2 Statistical Analysis

Analyses were performed using Statistica V 6.1 (Statsoft, Inc. Tulsa, OK). During the training session, the independent variables were treatment (control or taurine-fed animals) and training day, whereas the dependent variables were latency to fall, distance traveled, and animal velocity. For analysis of the testing day, the independent variables were treatment (control or taurine-fed animals) and rpm

speed, while the dependent variables were latency to fall, distance traveled, and animal velocity. Therefore, a multifactorial analysis of variance was used in order to study the interaction effects among treatments. Significance was set at a confidence level of 95%, and data are presented as mean ± SD.

15.3 Results

15.3.1 The Effects of Taurine on Latency to Fall

Control mice displayed longer latencies to fall and had substantial day-to-day improvements in performance when compared to taurine-fed animals (Fig. 15.1a). Two-way ANOVA (training day × treatment) on latency to fall showed a significant effect of training day ($F_{1,6} = 12.15$, $p < 0.01$), suggesting a learning component to rotarod performance. A main effect of treatment was also observed ($F_{1,6} = 12.86$, $p < 0.01$) showing that taurine significantly decreased latency to fall compared to control group performance. Therefore, the taurine-supplemented mice were not improving their performance over training days.

After training, mice were tested at eight different rpm speeds (Fig. 15.1b). Two-way ANOVA (rpm × treatment) on latency to fall revealed a main effect of rpm ($F_{1,7} = 18.45$, $p < 0.01$) with faster rotational speeds leading to shorter latency to fall. Also, an effect of treatment was observed ($F_{1,7} = 17.89$, $p < 0.01$) showing that taurine significantly decreased the latency to fall compared to controls. An rpm × treatment interaction was also found ($F_{1,7} = 3.03$, $p < 0.01$), indicating that the taurine-fed mice fell off earlier at higher rpm.

15.3.2 The Effects of Taurine on Distance Traveled

The taurine-fed mice displayed minor improvements in distance traveled after repeated training (Fig. 15.2a). A main effect of treatment was observed ($F_{1,6} = 100.09$, $p < 0.01$), indicating that the taurine-supplemented mice traveled shorter distances when compared to control animals. An effect of training day was also observed ($F_{1,6} = 17.85$, $p < 0.01$), and a training day × treatment interaction ($F_{1,6} = 9.14$, $p < 0.01$). This interaction suggests that control group distance traveled improved over training days, while taurine-fed mice displayed stable performance.

In Fig. 15.2b, the distance traveled is represented for both groups at different rpm speeds. Two-way ANOVA (rpm × treatment) on distance traveled revealed a main effect of treatment ($F_{1,7} = 36.32$, $p < 0.01$), implying that taurine-fed mice traveled shorter distances. A significant effect was also observed for rpm speed ($F_{1,7} = 2.38$, $p < 0.05$), with an rpm × treatment interaction ($F_{1,7} = 3.41$, $p < 0.01$). This interaction insinuates that taurine supplementation caused mice to travel shorter distances at faster rpm speeds.

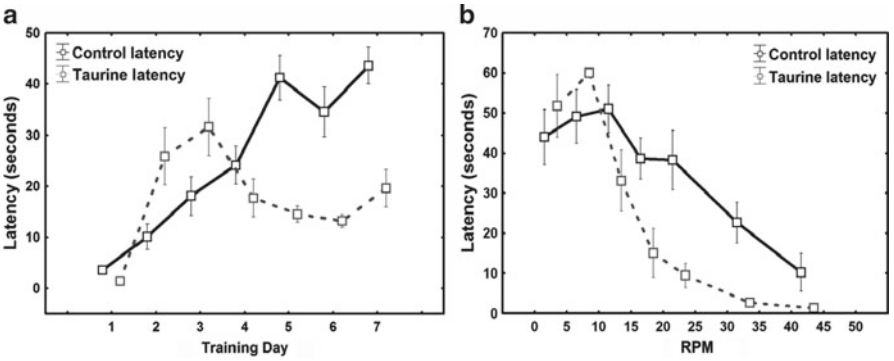

Fig. 15.1 The effects of taurine on rotarod performance when evaluating latency to fall. Taurine (0.05%) was supplemented in the drinking water for 4 weeks. Data represent mean ± SD. Control, $n=3$; taurine-fed, $n=3$. Mice were trained for 7 days (**a**). Control mice displayed longer latencies to fall and had substantial day-to-day improvements in performance when compared to taurine-fed animals. After training, mice were tested at eight different rpm speeds (**b**). Taurine-fed mice exhibited shorter latencies to fall at faster speeds

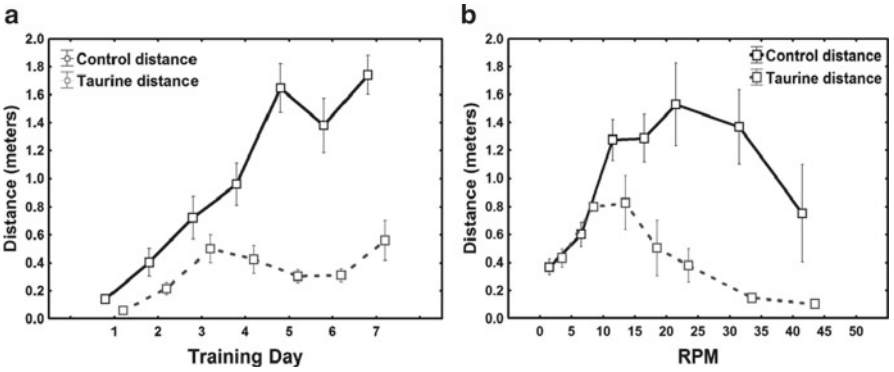

Fig. 15.2 Effects of taurine when assessing distance traveled on the rotarod apparatus. Taurine (0.05%) was supplemented in the drinking water for 4 weeks. Data represent mean ± SD. Control, $n=3$; taurine-fed, $n=3$. Mice were trained for 7 days (**a**). The taurine-fed mice displayed minor improvements after repeated training. During the testing phase (**b**) the taurine-fed mice demonstrated poorer performance as the ramp speed increased

15.3.3 The Effects of Taurine on Animal Velocity

Two-way ANOVA (training day × treatment) on animal velocity showed a main effect of treatment ($F_{1,6}=737.77$, $p<0.01$). The control animals exhibited stable performance over the course of training, while the taurine-fed mice showed minor improvements (Fig. 15.3a). A significant effect was also seen for training day ($F_{1,6}=30.45$, $p<0.01$), with a training day × treatment interaction effect ($F_{1,6}=38.65$, $p<0.01$).

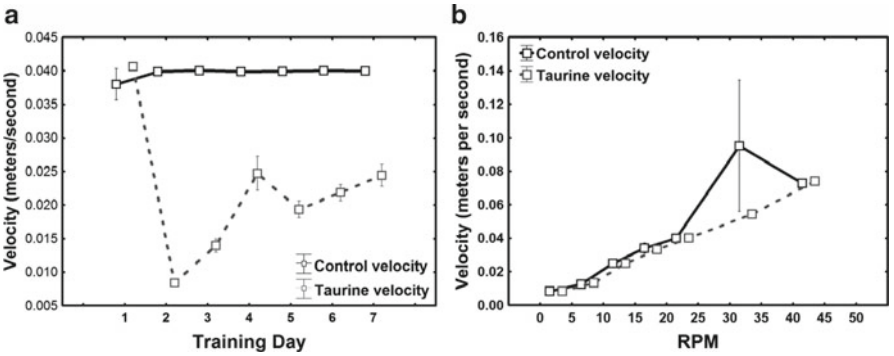

Fig. 15.3 The effects of taurine supplementation on movement velocity. The control animals exhibited stable performance over the course of training (**a**), while the taurine-fed mice showed minor improvements. During the testing phase (**b**), all animals were achieving similar velocities during the task

This interaction implies that taurine affected animal velocity with respect to training day.

In Fig. 15.3b, the animal velocity is depicted for both groups at each rpm speed during the testing phase. A two-way ANOVA (rpm × treatment) on animal velocity revealed an effect of rpm ($F_{1,7}=4.34$, $p<0.05$) with faster rotational speeds leading to increased velocities. However, the effect of treatment on animal velocity was nonsignificant ($F_{1,7}=0.943$, $p>0.05$), and the interaction effect was also nonsignificant ($F_{1,7}=1.00, p>0.05$). Therefore, taurine treatment did not significantly affect animal velocity. Taurine-fed and control animals did not differ significantly at each rpm speed.

15.4 Discussion

Motor coordination is a complex behavioral domain that can reflect balance, muscle strength, and patterned gait, as well as sensory competence. Difficulties in motor performance can confound behavioral assays of learning and memory, exploration, and motivation (Rustay et al. 2003). On the other hand, the learning of skilled movement is a finely tuned process that is dependent on interactions between the supplementary motor area, prefrontal parietal cortex, basal ganglia, and cerebellum. Given the neurocircuitry involved in motor learning and performance, one might expect to see differences in learning and performance on the rotarod among a set of inbred strains (Rustay et al. 2003), or after drug treatment. Results from this study suggest that there may be differences in the underlying structure and/or function of the brain regions involved in rotarod performance after chronic taurine supplementation.

Acquisition of a motor skill was examined after chronic taurine supplementation during the training phase of this study. The control animals continued to show

improvement in their ability to maintain balance on the rotarod apparatus (Fig. 15.1a). This was evident by their capacity to achieve longer latencies prior to falling in comparison to the taurine-fed mice. The results of this study suggest that chronic taurine supplementation affects the ability of the animal to coordinate and maintain balance since refinement was not observed over repeated training. Furthermore, the control animals displayed progressive improvement in the distance traveled from day to day, which is in contrast to taurine-fed animals.

During the testing phase as illustrated in Fig. 15.1b, an interaction effect can be seen with regard to chronic taurine supplementation and increased task difficultly. The control group exhibited subtle declines in the latency to fall when the rotarod speed was increased from 15 to 24 rpm. Beyond this speed, control animals were able to coordinate and maintain balance on the rotarod to a greater degree than taurine-fed animals. Additionally, the distance traveled by the control animals steadily inclined from 5 to 24 rpm (Fig. 15.2b). Unarguably, this makes sense since faster speeds at longer durations correlate nicely with distance traveled. Conversely, animals that received chronic taurine supplementation displayed poorer performances at speeds beyond 15 rpm.

Motor coordination is the ability of the organism to perform compound movements smoothly, whereas motor learning is the ability to adapt motor coordination to changes in task demands. Both processes are generally thought of as functions of the cerebellum and a deficit in either one of them could result in impaired performance on rotarod task parameters.

15.5 Conclusion

In summary, this study shows that taurine regulates motor learning behavior in mice. Our data show that chronic taurine supplementation may have contributed to motor learning deficits. The taurine-fed animals displayed minor improvements after repeated training when compared to controls. During the testing session the taurine-fed animals also exhibited a shorter latency to fall, as the task requirements became more demanding.

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