Published in final edited form as:

Cerebellum. 2014 June ; 13(3): 346–353. doi:10.1007/s12311-013-0541-4.

Glutamate dysfunction associated with developmental cerebellar damage: Relevance to autism spectrum disorders

Erik J. McKimm¹, Beau Corkill¹, Dan Goldowitz², Lorraine M. Albritton³, Ramin Homayouni⁴, Charles D. Blaha¹, and Guy Mittleman¹

¹Department of Psychology, The University of Memphis, 400 Innovation Drive, Memphis, TN 38152

²Centre for Molecular Medicine and Therapeutics, Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

³Microbiology, Immunology and Biochemistry, The University of Tennessee Health Science Center, 858 Madison Ave., Memphis, TN 38163

⁴Bioinformatics Program & Center for Translational Informatics, Dept. of Biology, The University of Memphis, 3774 Walker Ave, Memphis, TN 38152

Abstract

Neural abnormalities commonly associated with autism spectrum disorders include prefrontal cortex (PFC) dysfunction and cerebellar pathology in the form of Purkinje cell loss and cerebellar hypoplasia. It has been reported that loss of cerebellar Purkinje cells results in aberrant dopamine neurotransmission in the PFC which occurs via dysregulation of multisynaptic efferents from the cerebellum to the PFC. Using a mouse model we investigated the possibility that developmental cerebellar Purkinje cell loss could disrupt glutamatergic cerebellar projections to the PFC that ultimately modulate DA release. We measured glutamate release evoked by local electrical stimulation using fixed potential amperometry in combination with glutamate selective enzyme-based recording probes in urethane anesthetized Lurcher mutant and wildtype mice. Target sites included the medio-dorsal and ventro-lateral thalamic nuclei, reticulo-tegmental nuclei, pedunculopontine nuclei, and ventral tegmental area. With the exception of the ventral tegmental area, results indicated that in comparison to wildtype mice, evoked glutamate release was reduced in Lurcher mutants by between 9% to 72% at all stimulated sites. These results are consistent with the notion that developmental loss of cerebellar Purkinje cells drives reductions in evoked glutamate release in cerebellar efferent pathways that ultimately influence PFC dopamine release. Possible mechanisms whereby reductions in glutamate release could occur are discussed.

Keywords

Autism; Cerebellum; Dopamine; Glutamate; Fmr1 mice

Introduction

Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders that are diagnosed in young children generally before the age of six (American Psychiatric Association, 2000). Individuals diagnosed within this spectrum exhibit deficits in social skills, communication, and repetitive behavior, as well as various cognitive abnormalities including deficits in executive function [1, 2]. Prevalence rates of ASD are rising, making it imperative that insights into the biological bases of these disorders are discovered [2, 3].

There are a number of strategies that can be employed in order to uncover the biological underpinnings of ASD. One strategy that we have used is to concentrate on well documented and consistent evidence of brain damage associated with ASD. Cerebellar neuropathology including cerebellar hypoplasia and reduced cerebellar Purkinje cell numbers are the most consistent neuropathologies linked to ASD, although not all ASD patients exhibit reductions in cerebellar Purkinje cells at autopsy which may partly be related to premortem conditions and methodological differences in histological staining [5–14]. Coupled to this cerebellar neuropathology, abnormalities in another late-developing structure, the frontal cortex have also been reported. Quantitative MRI of autistic children revealed increased volume of the frontal lobe, which was correlated with the degree of cerebellar abnormality [15]. Using stereological methods on postmortem tissue it has also been reported that autistic male children average 67% more neurons in the frontal lobe, in comparison to non-autistic, male controls [16]. As measured by PET, autistic children also show low medial prefrontal cortex (mPFC) dopaminergic activity compared to age and gender matched controls [17].

Using a mouse model we have investigated how developmental damage of the cerebellum influences the appearance of autism-like symptoms and cognitive deficits, as well as the neural mechanisms by which these could occur. Lurcher mutant (Lc+) mice have an autosomal dominant mutation that results in a nearly complete loss of cerebellar Purkinje cells between the 2nd and 4th weeks of life [18, 19]. Since these mice are ataxic we used non-ataxic chimeric mice (Lc/+↔+/+), which have a variable loss of Purkinje cells dependent upon the incorporation of the wildtype lineage, to examine the behavioral impact of cerebellar Purkinje cell loss [20]. We found that: (1) Chimeric mice with reduced numbers of cerebellar Purkinje cells show exaggerated repetitive behavior and deficits in executive function that are significantly, negatively correlated with the number of cerebellar Purkinje cells obtained from cell counts [21, 22]. These results directed us to the mPFC because the occurrence of repetitive behavior and deficits in executive function has been linked directly to frontal cortical function [23, 24]. (2) cerebellar output through the cerebellar dentate nucleus (DN) modulates dopamine (DA) release in the mPFC via two independent pathways [25]. As shown in Figure 1, both of these pathways originate in the cerebellar cortex and then project to the DN. The first involves indirect activation of mesocortical dopaminergic neurons in the ventral tegmental area (VTA) via contralateral glutamatergic projections of the DN to reticulotegmental nuclei (RTN) that, in turn, project to pedunculopontine nuclei (PPT) and then project to, and stimulate directly, VTA dopaminergic cell bodies projecting to the mPFC [26–29]. The second pathway involves activation of the contralateral glutamatergic projections of the DN to thalamic mediodorsal and ventrolateral nuclei (ThN md and ThN vl) that send glutamatergic efferents to the mPFC

to modulate mesocortical dopaminergic terminal release in the mPFC via appositional excitatory glutamatergic synapses [30–33]. (3) In Lurcher mutant mice, we found that stimulating the cerebellar DN evokes mPFC DA release, although the DA response is significantly attenuated in comparison to Lurcher wildtype mice [25]. (4) In Lurcher wildtype mice, blocking glutamatergic transmission along either of these pathways reduced cerebellar dependent mPFC DA release by around 50% in each case, suggesting that the two pathways contribute equally and that they are primarily, if not entirely, glutamatergic [34]. (5) This 50% balance between the thalamic and VTA pathways in wildtype mice is altered in Lurcher mutants. In addition to the attenuation in cerebellar influence on the mPFC (point 3 above), there is a shift in the balance of influence of these pathways [35]. Thus, in Lurcher mutants the influence of the thalamic pathway is increased to 70% of the cerebellar signal while the VTA pathway is reduced to 30% of this signal. Considered together these findings suggest that cerebellar neuropathology in the form of Purkinje cell loss results in dysregulation of cerebellar neural circuitry modulating mPFC DA release, which can impinge on cognitive functions including repetitive behavior and executive function.

As projections from the DN to the thalamic nuclei and VTA are entirely glutamatergic it is reasonable that alterations in the functional properties of this neurotransmitter are driving the reductions in mPFC evoked dopamine release. Specifically, it is possible that only the first order projections from the DN to the RTN, and DN to ThN md and ThN vl are selectively affected, or alternatively, that there are generalized changes in glutamatergic responsiveness throughout both pathways that additionally encompass PPT and VTA. The purpose of this study was to distinguish between these possibilities. Thus, we evaluated glutamate release in RTP, PPT and VTA (VTA pathway) and ThN md and ThN vl (thalamic pathway) evoked by localized electrical stimulation using fixed potential amperometry in combination with glutamate enzyme-based recording probes in urethane anesthetized Lurcher wildtype and mutant mice in order to distinguish between these possibilities.

Methods

Animals

Experimental subjects were bred and maintained in the Animal Care Facility located in the Department of Psychology at the University of Memphis. Mice were continuously maintained in a temperature controlled environment ($21 \pm 1^\circ\text{C}$) on a 12:12 light:dark cycle (lights on at 0800) and were given free access to food and water. Original Lurcher (#001046) breeders were purchased from The Jackson Laboratory (Bar Harbor, Maine). All experiments were approved by a local Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Breeding

To produce Lurcher mutant (Lc+) mice, ataxic male mice heterozygous for the Lurcher spontaneous mutation (B6CBACa $A^{w-J}/A\text{-Grid2}^{Lc}$) were bred with non-ataxic female wildtype (WT) mice (B6CBACa $A^{w-J}/A\text{-Grid2}^{+}$). This breeding strategy produced litters composed of both heterozygous mutant and WT mice. Due to their ataxic gait, mice

heterozygous for the Lurcher mutation are easily distinguishable from their non-ataxic WT littermates.

Surgery

A total of 61 adult male Lc+ or WT mice were used. Six Lc+ and three WT mice were used for the assays in the ThN md, six Lc+ and six WT mice for the ThN vl, ten Lc+ and ten WT mice for the RTN, seven Lc+ and four WT mice for the VTA, and four Lc+ and five WT mice for the PPT. All were urethane anesthetized (1.5 g/kg i.p.) and placed in a stereotaxic frame. Body temperature was maintained at $36 \pm 0.5^\circ\text{C}$ with a temperature-regulated heating pad. Fixed potential amperometry (FPA) was used with a concentric bipolar stimulating electrode (SNE-100, Kopf Instruments) and a glutamate enzyme recording probe (model 7001, Pinnacle Technology), with an Ag/AgCl reference electrode component. In individual mice an electrode was implanted adjacent to the stimulating electrode in the left hemisphere using coordinates with respect to bregma and depth with respect to dura, in either the ThN md (AP -1.35 , ML $+0.4$, and DV -2.75), ThN vl (AP -1.35 , ML $+1.0$, and DV -3.45), RTN (AP -4.6 , ML $+3$, and DV -4.3), PPT (AP -4.7 , ML $+1.2$, and DV -2.75), or the VTA (AP -3.3 , ML $+0.35$, and DV -4.0). Figure 1 indicates the stimulation/recording sites.

Fixed potential amperometry and electrical stimulation

FPA coupled with glutamate enzyme-based recording probes has been shown to be a valid technique for real-time monitoring of stimulation-evoked glutamate release [36, 37]. Briefly, all glutamate recording probes were calibrated in a flow cell using glutamate solutions (10–400 μM) prior to insertion in the brain. A stock solution of L-glutamic acid (monosodium salt hydrate) and saline was freshly prepared prior to each probe calibration. Five separate concentration steps (10, 50, 100, 200, 400 μM) were injected sequentially through the flow cell over the recording probe. As *in vivo*, a fixed positive potential (+0.6 V) was applied to the recording probe and oxidation current was monitored continuously. Linear glutamate concentration-oxidation current correlation coefficients equal to or greater than 0.97 were deemed acceptable. Individual probe calibration values were used to establish glutamate concentrations recorded *in vivo*.

All recording sites were taken from [38]. A fixed positive potential (+0.6 V) was applied to the recording electrode and oxidation current was monitored continuously (10K samples/sec) by an electrometer filtered at 50 Hz. A total of 3 stimulations (100 monophasic 0.5 msec duration pulses at 100 Hz every 600 secs) were applied to the ThN md, ThN vl, RTN, PPT, or VTA (at 800 μAmps). As reviewed recently by [39], the spread of effective current is proportional to the square of the distance between the electrode tip and is highly dependent on the pulse duration and current–distance constant of neurons (low vs. high excitability threshold). Current–distance constants typically range from 100 $\mu\text{A mm}^2$ to 4000 $\mu\text{A mm}^2$ in brain tissue. Myelinated axons such as glutamatergic fibers exhibit a relatively high current–distance constant of 2000–4000 $\mu\text{A mm}^2$ [40]. Thus, for high-threshold neurons ($\sim 3500 \mu\text{A mm}^2$), we estimate that 0.5 ms pulses at 800 μA would activate these neuronal elements $\sim 0.6 \text{ mm}$ from the electrode tip. Six hundred seconds after the final stimulation the glutamate probe was withdrawn from the brain and a bipolar

stimulating electrode was introduced into the same location. A direct current (100 μ A for 10 s) was passed through this electrode to lesion the stimulation/recording site.

Data Analysis

Based upon probe calibration, oxidation current following each of the three stimulations was converted to evoked glutamate concentration (micromoles) at 5 second intervals ranging from 0 to 240 seconds post-stimulation. The initial mixed model analysis of variance involved four factors. Between subjects factors included Site (ThN md, ThN vl, RTN, PPT and VTA) and Group (WT or Lc+), while Stimulation (1, 2, 3) and Time (0 to 240 seconds in 5 second intervals) were the within subjects factors. Results of this initial, omnibus ANOVA necessitated additional 3 factor ANOVAs restricted to each stimulation site. When appropriate, between group differences were further analyzed using simple main effects tests or Dunnett's t-tests.

Results

Histology

Immediately following each experiment each mouse was euthanized with a lethal intracardial injection of urethane. Brains were removed and preserved in 10 % buffered formalin containing 0.1 % potassium ferricyanide, and then stored in 30 % sucrose/10 % formalin until sectioning. The brains were sectioned on a cryostat at -30°C . A Prussian blue spot indicative of the redox reaction of ferricyanide and iron deposits labeled the stimulating electrode tip in each stimulation/recording site. As shown in Figure 2, stimulation/recording sites were localized to the target sites. The ranges in localized histology were, in mm, (AP -1.22 to -1.46 , ML $.25$ to $.75$, DV -2.5 to -3.25 ; AP -1.06 to -1.46 , ML $.75$ to 1.25 , DV -3.25 to -3.75 ; AP -4.48 to -4.72 , ML $.20$ to $.75$, DV -4.0 to -4.6 ; AP -4.48 to -4.72 , ML $.75$ to 1.25 , DV -2.5 to -3.25 ; and AP -3.08 to -3.4 , ML $.2$ to $.6$, DV -3.75 to -4.25) for the ThN md, ThN vl, RTN, PPT, and VTA respectively.

Evoked Glutamate Release

Figure 3 shows the time course of stimulation-evoked glutamate release averaged across stimulations at the different sites. The omnibus ANOVA indicated that significant differences as occurred as a function of stimulation Site (Site: $F=3.44$, $df=4,51$, $p<.02$) and Group (Group: $F=8.44$, $df=1,51$, $p=.005$), and that group differences in the time course of evoked glutamate release occurred as a function of Site and Stimulation number (Site X Stimulation: $F=1.97$, $df=384,4752$, $p<.001$). Thus, each Site was subsequently analyzed separately. As shown in Figure 3, WT mice had significantly greater evoked glutamate release in the ThN md in comparison to Lc+ animals (Group X Time: $F=15.4$, $df=48,336$, $p<.001$). Dunnett's t-tests further indicate that groups differed significantly between 5 and 90 seconds post-stimulation. A similar pattern was observed in response to electrical stimulation of the ThN vl (Group X Time: $F=3.67$, $df=48,480$, $p<.001$), although the significantly increased glutamate release in WT mice persisted for up to 100 seconds post-stimulation (Dunnett's t-test). Lc+ and WT mice also differed significantly in evoked glutamate release in the RTN (Group X Time: $F=5.06$, $df=48,864$, $p<.001$), although this difference was shorter in duration and occurred from 5 to 70 seconds post-stimulation

(Dunnett's t-test). Group differences in evoked glutamate release were somewhat smaller in the PPT (Group X Time: $F=2.29$, $df=48,336$, $p<.001$) and were apparent between 10 and 45 seconds post-stimulation (Dunnett's t-test). As shown in Figure 3, evoked glutamate release in WT and Lc+ mice was comparable following stimulation of the VTA (Group X Time: $F=0.5$, $df=48,432$, $p=ns$). Table 1 shows the micromolar evoked glutamate release averaged across 3 stimulations over the 240 second recording intervals at each recording site in WT and Lc+ mice. As can be seen in this table, Lc+ mice had the largest reductions in glutamate release in ThN md, ThN vl and RTN.

Figure 4 shows the amount of evoked glutamate release for each of the 3 stimulations averaged over the 240 second post-stimulation recording interval. A number of different patterns of response were apparent over successive stimulations. The average amount of evoked glutamate release was maintained in WT and Lc+ animals following repeated electrical stimulations in the ThN md (Stimulation: $F=0.54$, $df=2,14$, $p=ns$), RTN (Stimulation: $F=0.19$, $df=2,34$, $p=ns$), or PPT (Stimulation: $F=2.30$, $df=2,14$, $p=ns$). In the ThN vl there was a group difference in evoked glutamate release (Group X Stimulation: $F=3.95$, $df=2,19$, $p<.05$). Simple main effects tests revealed that in WT mice, glutamate release increased significantly during the second and third stimulations in comparison to the first (Stimulation: $F=4.67$, $df=2,10$, $p<.05$). In contrast, in the ThN vl, glutamate release declined significantly across stimulations in Lc+ animals ($F=4.41$, $df=2,9$, $p<.05$). In the VTA there was a small but significant increase in evoked glutamate release across the 3 stimulations in both WT and Lc+ mice (Stimulation: $F=3.97$, $df=2, 18$, $p<.05$).

Discussion

The present results show that electrical stimulation of glutamatergic axons in the ThN md, ThN vl, RTN, and PPT of Lc+ mice resulted in significantly less evoked glutamate release than in WT control mice (Fig. 3). Evoked glutamate release was equivalent in the VTA of both genotypes. As such, these results are consistent with the idea that both first and second order glutamatergic projections from the DN are abnormal in Lc+ mice possessing developmental cerebellar neuropathology that involves loss of cerebellar Purkinje cells [18, 19]. It thus seems highly likely that it is this cerebellar neuropathology that drives the subsequent reduction in glutamate release observed in cerebellar efferents from the DN. As both pathways participate in the modulation of mPFC dopamine release, it appears reasonable that these reductions in glutamate release can account for the previously observed reduction in mPFC dopamine release elicited by electrical stimulation of the DN in Lc+, compared to WT, mice [25].

It is important to point out that the observed reductions in glutamate release in cerebellar efferent pathways could result from at least two different sources. As a function of loss of Purkinje cells in the cerebellar cortex and subsequent reductions in impulse traffic to the DN, it is possible that reductions in the population of glutamatergic efferents has occurred, which correspond to reductions in glutamate available for release. In support of this proposal, reductions in glutamate release have been shown to correlate with reductions in the numbers of activated glutamatergic neurons *in vivo*. The concentration of glutamate immunoreactivity and the concentration of glutamate neurons corresponded to associated

glutamate evoked neuronal activity in studies of the visual cortex in monkeys [41] and cats [42]. In schizophrenia, another disorder where diverse symptomatology that has been suggested to result from developmental abnormalities in prefrontal cortico-cerebellar-thalamo-prefrontal cortical circuits [43], cerebellar abnormalities including reductions in cerebellar Purkinje cells have been observed [44,45]. Diffusion tensor imaging in schizophrenics has revealed reduced anisotropic diffusion in connectivity between cerebellum and thalamus, which was interpreted as a loss of connectivity [46]. It seems reasonable that a reduction in the population of glutamatergic efferents from the DN to the thalamus, including the RTN and its projections to the PPT, could contribute to a loss in connectivity and drive. Indeed, quantitative morphological analysis of deep cerebellar nuclei have shown a 20% and 37% reduction in the number of principal and small neurons, respectively, in the DN of adult Lc+ mice as a result of secondary transneuronal degeneration brought on by the developmental loss of cerebellar Purkinje cells [47]. In addition, volumetric analysis has shown the loss of myelinated axons and boutons accounts for 59% of the atrophy of the DN in Lc+ mice, with the remaining 41% accounted for by a loss in nuclear neurons, dendritic arbors and reduced volume of glial processes, vascular elements, and intercellular space [48].

Alternatively, as a function of loss of Purkinje cells in the cerebellar cortex and subsequent reductions in impulse traffic to the DN, glutamatergic receptors and cellular machinery contributing to the processes of synthesis and release may be altered, resulting in a reduction in glutamatergic transmission and glutamate available for release. For example, autoradiography of glutamate receptor binding in adult Lc+ mice, compared to WT mice, has shown a significant decrease in ionotropic glutamatergic receptors in the DN, as well as in the thalamus, neostriatum, limbic regions, and cerebral cortical regions [49].

As shown in Fig. 5, glutamate can be generated in the mitochondria from glutamine by glutaminase 1 (Gls1 gene products) and then transported into presynaptic vesicles via transporters Vglut1/2 (Slc17a7 and Slc17a6 gene products) or further metabolized to α -ketoglutarate by glutamate dehydrogenase (GluD1 gene product) as part of cellular metabolism. During synaptic transmission, glutamate can bind to a number of ionotropic (NMDA and AMPA/kainate) and group I metabotropic (mGluR1/5) receptors in the postsynaptic cell, as well as group II (mGluR2/3) and group III (mGluR4, 6–8) metabotropic receptors in the presynaptic terminal. For instance, glutamate neurotransmission is inhibited by activation of group I and II metabotropic receptors resulting in a down-regulation of postsynaptic glutamate response or presynaptic glutamate release, respectively. In addition, glutamate is removed from the synaptic cleft by glial transporters EAAT1/Glt2 (Slc1a3 gene product) and EAAT2/GLAST (Slc1a2 gene product). In astrocytes, glutamate is converted to glutamine and transported out by SN1, where it can be taken up by neurons via GLNT and SAT2, and where it is converted back to glutamate by mitochondrial glutaminase (GLS1) in order to replenish the vesicular glutamate pool in the presynaptic terminal. Astrocytes also contain glutamate decarboxylase (GAD) that enzymatically converts glutamate to GABA [50]. Thus, a reduction in glutamate stores could arise from multiple intraneuronal or astrocytic metabolic pathways including an upregulation of astrocytic GAD activity. For example, [51] examined the anterior cingulate cortex and found that the protein levels of kidney-type glutaminase, but not those of the other enzymes, were significantly

lower in subjects with autism compared to controls. In addition, using proton magnetic resonance spectroscopy in two brain regions implicated in ASD (the basal ganglia and prefrontal cortex), investigators have recently shown marked reductions in glutamate, and glutamine in autistic patients [52]. Thus, it is conceivable that glutamate synthesis, and thus tissue content, may be diminished in astrocytes and/or glutamatergic terminals in first order and second order glutamatergic projections from the DN in Lc+ mice resulting in an overall reduction in stimulation-evoked glutamate release.

We suspect that mechanisms involving reductions in the population of glutamatergic efferents, as well as reductions in the processes governing synthesis and release are reflected in Lc+ mice. Specifically, in Lc+ mice in the ThN md, RTN and PPT we found clear evidence of reductions in evoked glutamate release that were maintained across successive stimulations. We believe this pattern of results is indicative of a reduction in the population of glutamatergic axons in these nuclei. In contrast, evoked glutamate release in Lc+ mice was lower than that of WT mice, and additionally declined significantly with repeated stimulation in the Thn vl. It seems reasonable that this pattern of change is additionally indicative of alterations in the processes contributing to synthesis and release as well as reduced numbers of glutamatergic axons projecting to this nucleus. It should be noted that relatively little is known about glutamatergic projections from the DN to thalamic and brainstem nuclei in Lc+ mice or ASD. Thus, further investigation of these possibilities is warranted.

Acknowledgments

This project was made possible by NINDS grant 1R01NS063009.

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Figure 1.

Neuronal circuitry underlying cerebellar modulation of medial prefrontal cortex (mPFC) dopamine neurotransmission. Cerebellar modulation of dopamine release in the mPFC may occur via polysynaptic inputs from deep cerebellar nuclei, such as the dentate nucleus, to dopamine-containing cells in the ventral tegmental area or via a monosynaptic input to thalamic (md, mediodorsal; vl, ventrolateral) projections making close appositions with dopamine terminals in the mPFC. Glutamatergic pathways are shown as gray arrows and the dopaminergic pathway as a dashed arrow. Black curved arrow represents mPFC feedback to cerebellum via pontine nuclei. Gray ovals correspond to stimulation-evoked glutamate recording sites. Nuclei abbreviations are shown in the ovals

Figure 2.

Representative coronal sections in Lurcher (Lc+) and wildtype (WT) mice illustrating placements (gray shaded areas) of stimulation/recording electrodes in the mediodorsal nucleus of the thalamus (ThN md), ventrolateral nucleus of the thalamus (ThN vl), reticulotegmental nucleus (RTN), pedunculopontine nuclei (PPT), and ventral tegmental area (VTA). Numbers correspond to mm from bregma. Placements of stimulating/recording overlapped in all groups. Sections were adapted from the mouse atlas of Franklin and Paxinos, (2001).

Figure 3.

The time-course of glutamate release evoked by electrical stimulation of the mediodorsal nucleus of the thalamus (ThN md), ventrolateral nucleus of the thalamus (ThN vl), reticulotegmental nucleus (RTN), pedunculopontine nuclei (PPT), or ventral tegmental area (VTA) in Lucher (Lc+) mutant mice or wildtype (WT) controls. A total of 3 stimulations (100 monophasic 0.5 msec duration pulses at 100 Hz every 600 secs) were applied to the ThN md, ThN vl, RTN, PPT, or VTA (at 800 μ Amps). Results averaged across the 3 stimulations are shown. Vertical lines indicate the standard error of the mean.

Figure 4.

Average glutamate release evoked by electrical stimulation of the mediodorsal nucleus of the thalamus (ThN md), ventrolateral nucleus of the thalamus (ThN vl), reticulotegmental nucleus (RTN), pedunclopontine nuclei (PPT), or ventral tegmental area (VTA) in Lucher (Lc+) mutant mice or wildtype (WT) controls. A total of 3 stimulations (100 monophasic 0.5 msec duration pulses at 100 Hz every 600 secs at 800 μAmps) were applied to each stimulation/recording site. Results of each stimulation are shown. Vertical lines indicate the standard error of the mean. * $p < 0.05$ successive stimulations vs. first stimulation in WT and Lc+ mice.

Figure 5.

Schematic representation of glutamate release at cerebellar synaptic junctions. Glutamate (glu) released from pre-synaptic glutamatergic neurons acts on post-synaptic and presynaptic neuronal metabotropic and ionotropic (NMDA and AMPA) receptors. See text for details. VGLut, vesicular glutamate transporter; mGluR, metabotropic glutamate receptors; AMPA, α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; EAAT, excitatory amino acid transporter; GAD, glutamate decarboxylase; GABA, γ -aminobutyric acid; SN1, glutamine transporter; SAT2, system amino acid transporter; GLNT, neutral amino acid transporter; GLS, glutaminase; GLUD1, glutamate dehydrogenase.

Table 1

Difference in evoked glutamate release in Lc+ and WT mice averaged across 3 stimulations*

Stimulation Site	WT	Lc+	%Change
ThN md	129.7	51.0	-60.7
ThN vl	220.5	62.8	-71.5
RTN	223.6	151.8	-32.1
PPT	109.0	99.0	-9.2
VTA	72.6	65.1	-10.3

* micromolar values shown