Published in final edited form as:

Synapse. 2011 November ; 65(11): . doi:10.1002/syn.20960.

Connecting the Dots of the Cerebro-Cerebellar Role in Cognitive Function: Neuronal Pathways for Cerebellar Modulation of Dopamine Release in the Prefrontal Cortex

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Abstract

Cerebellar involvement in autism, schizophrenia, and other cognitive disorders is typically associated with prefrontal cortical pathology. However, the underlying neuronal mechanisms are largely unknown. It has previously been shown in mice that stimulation of the dentate nucleus (DN) of the cerebellum evokes dopamine (DA) release in the medial prefrontal cortex (mPFC). Here, we investigated the neuronal circuitry by which the cerebellum modulates mPFC DA release. Fixed potential amperometry was used to determine the contribution of two candidate pathways by which the cerebellum may modulate mPFC DA release. In urethane anesthetized mice, DA release evoked by DN stimulation (100 Hz) was recorded in mPFC following local anesthetic lidocaine (0.02 μ g) or ionotropic glutamate receptor antagonist kynurenate (0.5 μ g) infusions into the mediodorsal or ventrolateral thalamic nucleus (ThN md; ThN vl), or the ventral tegmental area (VTA). Following intra-VTA lidocaine or kynurenate infusions, DA release was decreased by ~50%. Following intra-ThN md and ThN vl infusions of either drug, DA release was decreased by ~35% and 15%, respectively. Reductions in DA release following lidocaine or kynurenate infusions were not significantly different indicating that neuronal cells in the VTA and ThN were activated primarily if not entirely by glutamatergic inputs. The present study suggests that neuropathological changes in the cerebellum commonly observed in autism, schizophrenia, and other cognitive disorders could result in a loss of functionality of cerebellar-mPFC circuitry that is manifested as aberrant dopaminergic activity in the mPFC. Additionally, these results specifically implicate glutamate as a modulator of mPFC dopaminergic activity.

Keywords

amperometry; cerebellum; dentate nucleus; DA; thalamus; ventral tegmental area

INTRODUCTION

The cerebellum is heavily implicated in autism, schizophrenia, and other cognitive and affective disorders (Andreasen and Pierson, 2008; Palmen et al., 2004; Schmahmann et al., 1998 ; Stoodley, in press). For example, brains of autistic individuals have been shown to have histopathological abnormalities in this structure. Cerebellar hypoplasia and reduced Purkinje cell numbers are the most reproducible neural pathologies linked with autism (Palmen et al., 2004; Vargas et al., 2005; Whitney et al., 2008). How abnormalities in a structure traditionally linked to motor function, posture, and balance contribute to the cognitive deficits is unknown (Morton and Bastian, 2004).

Clues to how cerebellar abnormalities may contribute to the cognitive and affective deficits come from multiple sources. Recent imaging studies in primates and humans revealed strong functional connectivity between the cerebellum and prefrontal cortical areas (Krienen and Buckner, 2009). In mice and rats, recent research indicates that both Purkinje cell degeneration and lesions to deep cerebellar dentate nucleus (DN) result in visuospatial and sensorimotor learning deficits (Goodlett et al., 1992; Joyal et al., 2001; Noblett and Swain, 2003). Clinical studies have shown that cerebellar lesions and cerebellar atrophy result in cognitive and emotional impairments similar to those associated with autism (Botez-Marquard and Botez, 1993; Bracke-Tolkmitt et al., 1989; Schmahmann and Sherman, 1998). Developmental abnormalities or acute lesions of the cerebellum result in cognitive function deficits in associative learning, verbal ability, planning, and working memory which suggests that the cerebellum is directly involved in these functions (Bracke-Tolkmitt et al., 1989; Kumar et al., 2010; Rapoport et al., 2000; Schmahmann, 1991, 2001; Schmahmann and Sherman, 1998).

Structural abnormalities of the medial prefrontal cortex (mPFC) have been shown to exist in children with autism. The cortex of the frontal lobe is larger in individuals with autism, and increased volume of frontal lobe cortex has been demonstrated to be positively correlated with autistic symptoms and the degree of abnormality of the frontal cortex is correlated with the degree of abnormality in the cerebellum (Carper and Courchesne, 2000; Kumar et al., 2010). Recent functional magnetic resonance imaging (fMRI) studies also show a lower activity level within the mPFC of individuals with autism during a spatial working memory task (Luna et al., 2002). In addition, multi-voxel pattern analyses following the employment of fMRI have shown that the functional organization of the mPFC differs between those with and without autism (Gilbert et al., 2009). Diffusion tensor imaging of cerebellar pathways in autistic children suggest a reduced functional connectivity between the cerebellum and the cortex (Sivaswamy et al., 2010). Also, autistic and cerebellar-damaged patients have impaired performance as compared to controls on attention shifting tasks suggesting that abnormal functioning of the cerebellum might be responsible for some of the cognitive impairments observed in autism (Courchesne et al., 1994).

It is additionally noteworthy that dopamine (DA) release in the mPFC has been shown to be associated with autism (Ernst, 1997), schizophrenia (Bennett, 1998), emotional processing (Morrow et al., 1999), and a variety of cognitive functions including attention, working memory, and planning (Aalto et al., 2005; Gamo et al., 2010; Jackson and Mogdahham, 2004; Rose et al., 2010). Associated with frontal lobe damage, autistic patients also show deficits in representing their own or other's mental states including intentions, beliefs, wants, and knowledge, abilities that have been collectively referred to as "theory of mind" (Bird et al., 2004). These indices of higher cognitive function appear to be dependent on normal dopaminergic transmission in the mPFC (Lackner et al., 2010; Montague et al., 2004). For example, positron emission tomography (PET) studies have demonstrated that children with autism have reduced dopaminergic activity in the mPFC (Ernst et al., 1997).

Also, patients with hereditary degenerative ataxias due to cerebellar atrophy were found to have lower levels of the DA metabolite homovanillic acid in cerebrospinal fluid suggesting that overall DA extracellular concentrations are dependent on normal cerebellar function (Botez and Young, 2001).

Using mice heterozygous for the *lurcher* spontaneous mutation (*Grid2^{Lc}*), which have almost total loss of cerebellar Purkinje cells (Caddy and Biscoe, 1979; Zuo et al., 1997) and wildtype controls, we have recently shown that electrical stimulation of the cerebellar Purkinje cell layer and the DN is able to evoke the release of DA in the mPFC and that mPFC DA is dependent on functional cerebellar Purkinje cell outputs (Mittleman et al., 2008). This is in agreement with the disconnection hypothesis which posits that cognitive disorders like autism and schizophrenia result, at least in part, in an interruption of neuronal circuitry between these cerebral and cerebellar brain regions (Friston, 1999; Geshwind and Levitt, 2007). Thus, impaired connectivity between the cerebellum and mPFC could account for certain cognitive symptoms of autism.

Cerebellar-modulation of mPFC DA release could be mediated by two separate neuronal pathways originating in the DN, which serves as one of the principle deep cerebellar output nuclei of the cerebellum (see Fig. 3 inset). The first is by indirect activation of mesocortical dopaminergic neurons via contralateral projections of the DN to reticulo-tegmental nuclei (RTN) that, in turn, project to pedunculo-pontine nuclei (PPT) and then project to, and stimulate directly, ventral tegmental area (VTA) dopaminergic cell bodies projecting to the mPFC (Forster and Blaha, 2003; Garcia-Rill et al., 2001; Perciavalle et al., 1989; Schwarz and Schmitz, 1997; Snider et al., 1976). The second is by activation of the contralateral projections of the DN to thalamic mediodorsal/ventrolateral nuclei (ThN md/vl) that send efferents to the mPFC to modulate mesocortical dopaminergic terminal release in the mPFC via appositional excitatory glutamatergic synapses (Del Arco and Mora, 2005; Middleton and Strick, 2001; Pinto et al., 2003).

The aim of the present study was to determine the neuronal pathways involved in cerebellar modulation of mPFC DA release. To determine the relative contribution of each candidate neuronal circuit in modulating mPFC DA release, we used fixed potential amperometry in combination with carbon-fiber microelectrodes (Dickson et al., in press; Forster and Blaha, 2003; Lester et al., 2008) to monitor mPFC DA release evoked by DN electrical stimulation before and during individual infusions of the local anesthetic lidocaine, the ionotropic glutamate receptor antagonist kynurenic acid, and the control solution phosphate buffered saline (PBS) into the ThN md, ThN vl, or VTA of urethane anesthetized *lurcher* wildtype mice.

MATERIALS AND METHODS

Subjects

The original stock of *lurcher* mice, B6CBACa^{w-J}/*A-Grid2^{Lc}*, was obtained from the Jackson Laboratory in Bar Harbor, Maine. Forty-five *lurcher* wildtype mice bred at the University of Memphis were used in this study. The mice were housed five per cage and were 10–14 weeks of age at the time of surgery. The mice were maintained at the University of Memphis Animal Care Facility in a temperature controlled environment [(21.0 ± 1.0)°C] on a 12 h light:12 h dark cycle with food and water available ad libitum. 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.

Surgery

Mice were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame with head-holder adaptor to ensure that the skull was level. Body temperature was maintained at $(36 \pm 0.5)^{\circ}\text{C}$ with a temperature-regulated heating pad. Four holes were drilled into the animals' skulls to allow for the implantation of an Ag/AgCl reference/auxiliary combination electrode, a carbon-fiber microelectrode (DA recording electrode; carbon fiber 10 μm o.d., 250 μm length, Thorne Type P, Union Carbide, PA; Forster and Blaha, 2003), a concentric bipolar stimulating electrode (CBARD75, 125 μm outer and 25 μm inner pole diam., FHC, ME), and a 31 g stainless-steel guide cannula for drug microinfusions into appropriate nuclei. The Ag/AgCl reference/auxiliary combination electrode was placed contralateral to the recording electrode which was placed in mPFC of the left hemisphere at 30° lateral to medial angle (coordinates from bregma: AP +2.35 mm, ML +1.0 mm, DV -1.5 mm from dura; Paxinos and Franklin, 2001). The stimulating electrode was placed in the right DN (coordinates from bregma: AP -6.24 mm, ML -2.1 mm, DV -2.25 mm from dura; Paxinos and Franklin, 2001). The guide cannula tip was placed 1 mm above in the VTA, ThN md, or ThN vl (coordinates from bregma: AP -3.3 mm, ML +0.35 mm, DV -3.0 mm from dura; AP -1.35 mm, ML +0.4 mm, DV -2.75 mm from dura; AP -1.35 mm, ML +1.0 mm, DV -3.45 mm from dura, respectively; Paxinos and Franklin, 2001).

Fixed potential amperometry and electrical stimulations

Immediately following electrode implantation, a constant voltage of +0.8 V was applied to the recording electrode and oxidation current sampled continuously (10,000 samples/s) via an electrometer (ED401 e-corder 401 and EA162 Picostat, eDAQ, CO) filtered at 50 Hz (Forster and Blaha, 2003). Electrical stimulation of the DN consisted of 100 cathodic monophasic pulses (200–400 μA intensity, 0.5 ms pulse duration) at 50 Hz every 60 s for a period of 10 to 15 min and were applied to the stimulating electrode via an optical isolator and programmable pulse generator (Iso-Flex/Master-8; AMPI, Jerusalem, Israel).

Pathway inactivations

Following a baseline recording of at least 5 min, separate groups of mice received microinfusions of lidocaine (0.02 μg), kynurenate (0.5 μg), or PBS. Infusions were administered via the guide cannula. Drugs were first back-loaded into a fiberglass infusion cannula (80 μm o.d., Polymicro Tech., AZ) and then connected via PE10 tubing to a 1.0 μl microsyringe (Scientific Glass Engineering, TX). The infusion cannula was then placed into the guide cannula to extend 1 mm from its tip into the injection site. A 0.5 μl infusion of lidocaine, kynurenate, or PBS was then administered over 1.0 min period and left in place. Changes in DN stimulation-evoked DA oxidation current in the mPFC were recorded for 10 min postinfusion.

Data analyses

The three DN stimulation-evoked responses immediately prior to each drug infusion as well as the three responses immediately following infusion were extracted from the continuous record and amperometric currents within the range of 0.2 s prestimulation and 60 s poststimulation were normalized to zero current values. The currents for each evoked response were then summed across time (-0.2 s through 60 s) for each response due to DA concentrations significantly differing from prestimulation baseline for several seconds poststimulation (Mittleman et al., 2008). The relatively slow recovery of the evoked DA signal to prestimulation baseline is consistent with the paucity of DA transporters compared to the striatum (Sesack et al., 1998), a region more densely innervated with dopaminergic neurons (Palkovits, 1979). As the preinfusion responses and the postinfusion responses were each one minute apart, two repeated measures analyses of variance (ANOVA) were

conducted with time (1–3) of each preinfusion response and time (1–3) of each postinfusion response, each as a within-groups factor. For both analyses, site of infusion and drug administered were between-group factors and the response sums at each time point were the dependent variable. These analyses indicated that the stimulation-evoked responses did not differ significantly across either preinfusion or postinfusion times ($F(2, 72) = 1.63, P = 0.203$; $F(2, 72) = 0.056, P = 0.945$). Because the stimulation-evoked responses did not vary significantly, the three preinfusion response current sum values were averaged together to compare with the average of the three postinfusion response current sum values. Thus, the average percent postinfusion decrease was then obtained for each mouse with respect to preinfusion baseline responses (0%).

An ANOVA was then conducted using site of infusion (VTA, ThN md, or ThN vl) and drug/vehicle infused (lidocaine, kynurenate, or PBS) as between-groups factors and percent decrease of mPFC DA release as the dependent variable. If the ANOVA indicated a significant interaction between site of infusion and drug infused, Sidak–Bonferroni post-hoc comparisons were performed. Depending on the outcome of the post-hoc tests, a series of t -tests with a Bonferroni correction were performed to further identify significant differences.

Histology

Immediately following each experiment, a direct current (100 μ A for 10 s; +5 V for 5 s) was passed through the stimulating electrode in the DN to leave iron deposits and through the recording electrode in the mPFC to lesion tissue, respectively. Each mouse was then euthanized with a lethal intracardial injection of urethane. The brains were removed and preserved overnight in 10% buffered formalin containing 0.1% potassium ferricyanide, and then stored in 30% sucrose/10% formalin solution until sectioning. At the conclusion of the experiment, 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 the DN, while placements of the recording electrodes in the mPFC were determined by the position of the electrolytic lesion. Placements of the electrodes were confirmed under light microscopy and recorded on representative coronal diagrams (Paxinos and Franklin, 2001).

RESULTS

Stereotaxic placements of electrodes

Figure 1 depicts the central placements of stimulating and recording electrodes and infusion cannula tips. The stimulating electrode tips were confined within the DN ($n = 45$ electrodes, ranging from, in mm, -6.1 to -6.35 AP, $+1.9$ to $+2.2$ ML, and -2.1 to -2.6 DV) posterior to bregma, lateral to midline, and ventral from dura. Infusion cannula tips were confined to the ThN md ($n = 15$; ranging from in mm: -1.25 to -1.5 AP, $+0.2$ to $+0.5$ ML, and -2.45 to -2.95 DV), the ThN vl ($n = 15$; ranging from in mm, -1.2 to -1.45 AP, $+0.9$ to $+1.2$ ML, and -3.1 to -3.6 DV), and the VTA ($n = 15$; ranging from in mm: -3.1 to -3.4 AP, $+0.15$ to $+0.5$ ML, and -3.7 to -4.25 DV) posterior to bregma, lateral to midline, and ventral from dura. Recording electrode surfaces were confined within the mPFC ($n = 45$; ranging from in mm: $+2.2$ to $+2.4$ AP, $+0.75$ to $+1.2$ ML, and -1.9 to -2.1 DV) anterior to bregma, lateral to midline and ventral from dura.

Effects of infusions on stimulation-evoked DA release in mPFC

Both lidocaine and kynurenate infusions at each of the injection sites achieved their maximal inhibitory effects on DN stimulation-evoked DA release in the mPFC within 1 min of infusion. Figure 2 shows individual examples of mPFC DA release responses evoked by electrical stimulation of the DN, 3 min before and 3 min after infusions of PBS into the

VTA, and kynurenate or lidocaine into the ThN md. Prior to kynurenate, lidocaine, or PBS infusions, DN stimulation-evoked an increase in DA release with the signal peaking within ~5.5 s from the start of stimulation and returning to prestimulation baseline within 50–60 s (Fig. 2, left panels). A similar magnitude and time course in DN stimulation-evoked DA release in the mPFC was observed following PBS infusion into the VTA (Fig. 2, top, right panel). In contrast, DN stimulation-evoked release of mPFC DA was markedly attenuated as shown in the examples of an intra-ThN md kynurenate and lidocaine infusions with DA release peaking within ~2 to 5 s from the start of stimulation and returning to prestimulation baseline within 20–30 s (Fig. 2 middle and bottom, right panels). An ANOVA indicated a significant main effect for drug and site of infusion ($F(2, 36) = 65.53, P < 0.001$; $F(2, 36) = 28.53, P < 0.001$, respectively) as well as a significant interaction between drug and site of infusion ($F(4, 36) = 6.04, P = 0.001$). Sidak–Bonferroni post-hoc comparisons indicated that the average percent decrease of DN stimulation-evoked mPFC DA release in the three groups receiving PBS ($M = 1.9\% \pm 0.7\%$) did differ significantly from the average response of the groups receiving either lidocaine or kynurenate. However, the average percent decrease of DN stimulation-evoked mPFC DA release across all three groups receiving lidocaine ($M = 34.2\% \pm 4.8\%$) did not differ significantly from that of the three groups receiving kynurenate ($M = 33.3\% \pm 4.5\%$).

A series of *t*-tests with Bonferroni correction for multiple comparisons indicated that the percent decrease of mPFC DA release in each group following lidocaine or kynurenate infusions into the VTA, ThN md, and ThN vl were significantly different than the associated PBS controls ($P < 0.008$). In contrast, another series of *t*-tests with Bonferroni correction indicated that the percent decrease of mPFC DA release in each group following lidocaine was not significantly different than the kynurenate infusions into the VTA, ThN md, and ThN vl ($P > 0.05$). As shown in Figure 3, the site-specific average percent decrease for groups receiving lidocaine in the VTA, ThN md, and ThN vl were $51.8\% \pm 6.9\%$, $35.6\% \pm 4.7\%$, and $15.2\% \pm 1.6\%$, respectively. Similarly, kynurenate infusions resulted in comparable site-specific average percent decreases of $49.2\% \pm 6.2\%$, $35.4\% \pm 4.4\%$, and $15.3\% \pm 2.4\%$ for VTA, ThN md, and ThN vl, respectively. In contrast, PBS infusions resulted in relatively minimal site-specific average percent decreases of $2.8\% \pm 1.3\%$, $2.0\% \pm 1.1\%$, and $0.8\% \pm 1.3\%$ for VTA, ThN md, and ThN vl, respectively. As shown in Figure 3, the DN to mPFC pathway via dopaminergic neuronal cell bodies in the VTA was decreased by either lidocaine or kynurenate by ~50%, while the DN to mPFC via thalamic nuclei was also decreased similarly by either lidocaine or kynurenate by ~50% (35% in the ThN md and 16% in the ThN vl). Thus, the sum of the percent decreases occurring in each of these two pathways accounted for 100% of stimulation-evoked DA release recorded in the mPFC.

DISCUSSION

The present results extend our previous findings that cerebellar or DN electrical stimulation modulates mPFC DA release (Mittleman et al., 2008) by showing that this modulation is dependent on two discrete and independent cerebellar to mPFC neuronal circuits. Infusions of the local anesthetic lidocaine or the broad spectrum ionotropic glutamate receptor antagonist kynurenate into the ThN md, ThN vl, or VTA resulted in significant decreases in DN stimulation-evoked mPFC DA release, compared to PBS control infusions in the same anatomical sites. Figure 3 (inset) shows the underlying neuronal circuitry involved in deep cerebellar nuclei modulation of mPFC DA release. Thus, one circuit utilizes a DN-RTN-PPT-VTA-mPFC circuit (Forster and Blaha, 2003; Garcia-Rill et al., 2001; Schwarz and Schmitz, 1997) that involves activation of mesocortical dopaminergic transmission at the level of dopaminergic cell bodies in the VTA that synapse onto mPFC pyramidal neurons (Palkovits, 1979). In addition, the present results also indicate that DN stimulation-evoked

DA release in the mPFC may alternatively utilize a DN-ThN md/vl-mPFC circuit (Middleton and Strick, 2001; Pinto et al., 2003) that involves enhancement of mesocortical dopaminergic transmission via appositional glutamatergic synapses with DA neuronal terminals on mPFC pyramidal neurons (Del Arco and Mora, 2005). As such, these results provide the first demonstration of glutamatergic modulation of mPFC DA release via the cerebellar DN. The sum of the average percent decrease in mPFC DA release following infusions into the two thalamic nuclei is approximately equivalent to the decrease following infusions into the VTA suggesting that these two neuronal circuits contribute equally to cerebellar mPFC DA modulation (see Fig. 3 inset). In addition, as the average percent decreases of DA release following either lidocaine or kynurenate infusions in the VTA, ThN md, and ThN vl summed to ~100%, it would appear that cerebellar modulation of mPFC DA release may be wholly dependent on these two neuronal circuits. Also, attenuations in DN stimulation-evoked mPFC DA release in the groups receiving lidocaine and kynurenate were not significantly different which suggests that inputs into the VTA and ThN md/vl that contribute to mPFC DA release following DN stimulation are primarily, if not entirely, glutamatergic.

Abnormal levels of DA in the prefrontal cortex have been found to be associated with autism (Ernst et al. 1997), schizophrenia (Bennett, 1998), emotional processing (Morrow et al., 1999), and cognitive functions (Brozoski et al., 1979). Our finding of cerebellar modulation of DA in the PFC might thus provide a functional explanation of cerebellar involvement in autism (Sivaswamy et al., 2010), schizophrenia (Andreasen and Pierson, 2008), and other emotional and cognitive functions (Schmahmann and Sherman, 1998; Stoodley, in press). The equivalent participation of both neuronal circuits, instead of only one, may offer a greater degree of flexibility in cerebellar modulation of mPFC DA release, where an increase or decrease in activity in one of these circuits may be compensated for by the other. As well, other sources of inputs to subnuclei within a particular circuit, for example cortical and subthalamic inputs to the PPT (Hammond et al., 1983; Parent and Hazrati, 2001), could offer a means of fine tuning cerebellar outflow to the cortex and subsequent DA release. Indeed, it is important to note that the thalamus is a critical relay-station of the diencephalon through which nearly all sensory information is relayed to the cortex. It has input from striatum, globus pallidus, subthalamic nucleus, substantia nigra compacta/reticulata, and VTA. These inputs are integrated within the thalamus and conveyed to the cortex which, in turn, provides positive feedback to the thalamus for relevant input and suppresses irrelevant information (Da Cunha et al., 2009). The involvement of thalamic circuitry, by which the cerebellum modulates cortical DA release, would allow various inputs to the thalamus to modulate thalamic output to provide a more precise modulation of cortical DA release. The present study suggests that disconnections of cerebellar-mPFC circuitry, such as a reduction in the number of cerebellar Purkinje cells leading to disruption of information outflow from deep cerebellar nuclei, could result in aberrant dopaminergic activity in the mPFC. This notion supports the autism disconnection hypothesis and provides a neuroanatomical substrate via which the diversity of symptoms known to characterize autism could be mediated (Geschwind and Levitt, 2007).

Functional significance

Although this study did not address directly a loss of cerebellar Purkinje cells and resulting behavioral consequences of such a loss, previous clinical and preclinical studies suggest that cerebellar modulation of mPFC DA release is functionally relevant. For example, several clinical functional imaging and lesion studies which support the hypothesis that cerebellum and its reciprocal connections to the mPFC may contribute to the implementation of many executive functions (Bracke-Tolkmitt et al., 1989; Schmahmann and Sherman, 1998). In addition, using wildtype ↔ lurcher chimera mice that exhibit varying Purkinje cell counts

from 0 to 100%, we have previously shown that a developmental loss of Purkinje cells can produce a decrease in reversal learning in mice (Dickson et al., 2010). The results of this recent study indicated that cerebellar Purkinje cell number was directly correlated with impairments in behavioral flexibility in these mice. We have also previously shown in *lurcher* chimera mice that a developmental loss of Purkinje cells produces hyperactivity and repetitive behaviors as measured by activity and investigatory nose-poking during open-field activity and hole-board exploration tests (Dickson et al., 2010; Martin et al., 2004, 2006, 2010). Moreover, in rhesus monkeys bilateral electrolytic lesions of the DN results in increased perseveration during a conceptual set shifting task modeled after the Wisconsin card sorting task, suggesting that the output of the cerebellum, and specifically the DN, is essential for executive function mediated by the mPFC (Schmahmann et al., 2004). These executive function impairments mimic those seen in individuals with autism suggesting that certain cognitive deficits in autism are a result of aberrant dopaminergic activity due to a disconnection of cerebellar-mPFC circuitry.

CONCLUSIONS

The present findings suggest that both DN-RTN-PPT-VTA-mPFC and DN-ThN-mPFC neuronal circuitry contribute equally to cerebellar modulation of mPFC DA release. Based on previous research, this modulation appears to have functional relevance, such that the cerebellar Purkinje cell output modulates executive functions that parallel those cognitive functions which are impaired in individuals with autism, schizophrenia, and cerebellar cognitive affective syndrome. Our findings support the disconnection hypothesis which purports that cognitive disorders like autism and schizophrenia are due, at least in part, to reduced functional connectivity between brain structures, including the cerebellum and prefrontal cortex (Friston, 1999; Geschwind and Levitt, 2007). In future studies it will be important to identify how these circuits might compensate for a developmental loss of Purkinje cell output similar to that observed clinically. This issue could be experimentally addressed using a mouse model of variable Purkinje cell loss, such as *lurcher* chimeric mice, in order to determine how Purkinje cell number influences DA release in the mPFC and glutamatergic neurotransmission at different levels of cerebellar-mPFC circuitry. These studies would provide more detailed information as to the relationship between neuropathologies of the cerebellum and disruption of cerebellar-frontal cortex circuitry as well as suggesting potential new targets for pharmacological intervention.

Acknowledgments

The authors thank Tom Schneider for technical assistance with these experiments.

Contract grant sponsor: NINDS; Contract grant number: R01 NS063009

REFERENCES

- Aalto S, Bruch A, Laine M, Rinne J. Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: A positron emission tomography study using the high-affinity dopamine D2 receptor ligand [¹¹C]FLB 457. *J Neurosci*. 2005; 25:2471–2477. [PubMed: 15758155]
- American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV-TR. Author; Washington, DC: 2000.
- Andreasen NC, Pierson R. The role of the cerebellum in schizophrenia. *Biol Psychiatry*. 2008; 64:81–88. [PubMed: 18395701]
- Bennett MR. Monoaminergic synapses and schizophrenia: 45 years of neuroleptics. *J Psychopharmacol*. 1998; 12:289–304. [PubMed: 10958257]

- Bird CM, Castelli F, Malik O, Frith U, Husain M. The impact of extensive medial frontal lobe damage on 'Theory of Mind' and cognition. *Brain*. 2004; 127:914–928. [PubMed: 14998913]
- Botez MI, Young SN. Biogenic amine metabolites and thiamine in cerebrospinal fluid in hereditary degenerative ataxias. *Can J Neurol Sci*. 2001; 28:134–140. [PubMed: 11383938]
- Botez-Marquard T, Botez MI. Cognitive behavior in hereditary degenerative ataxias. *Eur Neurol*. 1993; 33:351–357. [PubMed: 8243509]
- Bracke-Tolkmitt R, Linden A, Canavan AGM, Rockstroh B, Scholz E, Wessel K, Diener H-C. The cerebellum contributes to mental skills. *Behav Neurosci*. 1989; 103:442–445.
- Caddy KW, Biscoe TJ. Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. *Phil Trans Biol Sci*. 1979; 287:167–201.
- Carper RA, Courchesne E. Inverse correlation between frontal lobe and cerebellum sizes in children with autism. *Brain*. 2000; 123:836–844. [PubMed: 10734014]
- Centers for Disease Control and Prevention. MMWR surveillance summary: Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States, 2006. 2009. Retrieved from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5810a1.htm>
- Courchesne E, Townsend J, Akshoomoff NA, Saitoh O, Yeung-Courchesne R, Lincoln AJ, James HE, Haas RH, Schreibman L, Lau L. Impairment in shifting attention in autistic and cerebellar patients. *Behav Neurosci*. 1994; 108:848–865. [PubMed: 7826509]
- Da Cunha C, Wietzikoski EC, Dombrowski P, Bortolanza M, Santos LM, Boschen SL, Miyoshi E. Learning processing in the basal ganglia: A mosaic of broken mirrors. *Behav Brain Res*. 2009; 133:157–170. [PubMed: 18977393]
- Del Arco A, Mora F. Glutamate-dopamine in vivo interaction in the prefrontal cortex modulates the release of dopamine and acetylcholine in the nucleus accumbens of the awake rat. *J Neural Transm*. 2005; 112:97–109. [PubMed: 15599608]
- Dickson PE, Rogers TD, Del Mar N, Martin LA, Heck D, Blaha CD, Goldowitz D, Mittleman G. Behavioral flexibility in a mouse model of developmental cerebellar Purkinje cell loss. *Neurobiol Learn Mem*. 2010; 94:220–228. [PubMed: 20566377]
- Dickson PE, Rogers TD, Lester DB, Miller MM, Matta SG, Chesler EJ, Goldowitz D, Blaha CD, Mittleman G. Genotype-dependent effects of adolescent nicotine exposure on dopamine functional dynamics in the nucleus accumbens shell in male and female mice: A potential mechanism underlying the gateway effect of nicotine. *Psychopharmacology*. (in press).
- Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM. Low medial pre-frontal dopaminergic activity in autistic children. *Lancet*. 1997; 350:638–639. [PubMed: 9288051]
- Forster GL, Blaha CD. Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and pons of the rat. *Eur J Neurosci*. 2003; 17:751–762. [PubMed: 12603265]
- Friston KJ. Schizophrenia and the disconnection hypothesis. *Acta Psychiatr Scand Suppl*. 1999; 395:68–79. [PubMed: 10225335]
- Gamo NJ, Wang M, Arnsten AF. Methylphenidate and atomoxetine enhance prefrontal function through $\alpha 2$ -adrenergic and dopamine D1 receptors. *J Am Acad Child Adolesc Psychiatry*. 2010; 49:1011–1023. [PubMed: 20855046]
- Garcia-Rill E, Skinner RD, Miyazato H, Homma Y. Pedunculopontine stimulation induces prolonged activation of pontine reticular neurons. *Neuroscience*. 2001; 104:455–465. [PubMed: 11377847]
- Geshwind DH, Levitt P. Autism spectrum disorders: Developmental disconnection syndromes. *Curr Opin Neurobiol*. 2007; 17:103–111. [PubMed: 17275283]
- Gilbert SJ, Meuwese JDI, Towgood KJ, Frith CD, Burgess PW. Abnormal functional specialization within medial prefrontal cortex in high-functioning autism: A multi-voxel similarity analysis. *Brain*. 2009; 132:869–878. [PubMed: 19174370]
- Goodlett CR, Hamre KM, West JR. Dissociation of spatial navigation and visual guidance performance in Purkinje cell degeneration (pcd) mutant mice. *Behav Brain Res*. 1992; 47:129–141. [PubMed: 1590945]
- Hammond C, Rouzair-Dubois B, Feger J, Jackson A, Crossman AR. Anatomical and electrophysiological studies on the reciprocal projections between the subthalamic nucleus and nucleus tegmenti pedunculopontinus in the rat. *Neuroscience*. 1983; 9:41–52. [PubMed: 6308507]

- Jackson ME, Moghaddam B. Stimulus-specific plasticity of prefrontal cortex dopamine neurotransmission. *J Neurochem*. 2004; 88:1327–1334. [PubMed: 15009632]
- Joyal CC, Strazielle C, Lalonde R. Effects of dentate nucleus lesions on spatial and postural sensorimotor learning in rats. *Behav Brain Res*. 2001; 122:131–137. [PubMed: 11334643]
- Krienen FM, Buckner RL. Segregated fronto-cerebellar circuits revealed by intrinsic functional connectivity. *Cerebral Cortex*. 2009; 19:2485–2497. [PubMed: 19592571]
- Kumar A, Sundaram SK, Sivaswamy L, Behen ME, Makki MI, Ager J, Janisse J, Chugani HT, Chugani DC. Alterations in frontal lobe tracts and corpus callosum in young children with autism spectrum disorder. *Cerebral Cortex*. 2010; 20:2103–2113. [PubMed: 20019145]
- Lackner CL, Bowman LC, Sabbagh MA. Dopaminergic functioning and preschooler's theory of mind. *Neuropsychologia*. 2010; 48:1767–1774. [PubMed: 20206642]
- Lester DB, Miller AD, Pate TD, Blaha CD. Midbrain acetylcholine and glutamate receptors modulate accumbal dopamine release. *Neuroreport*. 2008; 19:991–995. [PubMed: 18521007]
- Luna B, Minshew NJ, Garver KE, Lazar NA, Thulborn KR, Eddy WF, Sweeney JA. Neocortical system abnormalities in autism: An fMRI study of spatial working memory. *Neurology*. 2002; 59:834–840. [PubMed: 12297562]
- Martin LA, Escher T, Goldowitz D, Mittleman G. A relationship between cerebellar Purkinje cells and spatial working memory demonstrated in a lurcher/chimera mouse model system. *Genes Brain Behav*. 2004; 3:158–166. [PubMed: 15140011]
- Martin LA, Goldowitz D, Mittleman G. Sustained attention in the mouse: A study of the relationship with the cerebellum. *Behav Neurosci*. 2006; 120:477–481. [PubMed: 16719710]
- Martin LA, Goldowitz D, Mittleman G. Repetitive behavior and increased activity in mice with Purkinje cell loss: A model for understanding the role of cerebellar pathology in autism. *Eur J Neurosci*. 2010; 31:544–555. [PubMed: 20105240]
- Middleton FA, Strick PL. Cerebellar projections to the prefrontal cortex of the primate. *J Neurosci*. 2001; 21:700–712. [PubMed: 11160449]
- Mittleman G, Goldowitz D, Heck DH, Blaha CD. Cerebellar modulation of frontal cortex dopamine efflux in mice: Relevance to autism and schizophrenia. *Synapse*. 2008; 62:544–550. [PubMed: 18435424]
- Montague PR, Hyman SE, Cohen JD. Computational roles for dopamine in behavioral control. *Nature*. 2004; 431:760–767. [PubMed: 15483596]
- Morrow BA, Elsworth JD, Rasmussen AM, Roth RH. The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. *Neuroscience*. 1999; 92:553–564. [PubMed: 10408604]
- Morton SM, Bastian AJ. Cerebellar control of balance and locomotion. *Neuroscientist*. 2004; 10:247–259. [PubMed: 15155063]
- Noblett KL, Swain RA. Pretraining enhances recovery from visuospatial deficit following cerebellar dentate nucleus lesion. *Behav Neurosci*. 2003; 117:785–798. [PubMed: 12931963]
- Palkovits, M. The neurobiology of dopamine. Academic Press; London: 1979. p. 343–356.
- Palmen SJ, van Engeland H, Hof PR, Schmitz C. Neuropathological findings in autism. *Brain*. 2004; 127:2572–2583. [PubMed: 15329353]
- Parent A, Hazrati LN. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res Rev*. 2001; 20:91–127. [PubMed: 7711769]
- Paxinos, G.; Franklin, KBJ. The mouse brain in stereotaxic coordinates. 2nd ed. Academic Press; San Diego, CA: 2001.
- Perciavalle V, Berretta S, Raffaele R. Projections from the intracerebellar nuclei to the ventral midbrain tegmentum in the rat. *Neuroscience*. 1989; 29:109–119. [PubMed: 2469037]
- Pinto A, Jankowski M, Sesack SR. Projections from the paraventricular nucleus of the thalamus to the rat prefrontal cortex and nucleus accumbens shell: Ultrastructural characteristics and spatial relationships with dopamine afferents. *J Comp Neurol*. 2003; 459:142–155. [PubMed: 12640666]
- Rapoport M, van Reekum R, Mayberg H. The role of the cerebellum in cognition and behavior: A selective review. *J Neuropsychiatry Clin Neurosci*. 2000; 12:193–198. [PubMed: 11001597]

- Rose J, Schiffer A-M, Dittrich L, Güntürkün O. The roles of dopamine in maintenance and distractibility of attention in the “prefrontal cortex” of pigeons. *Neuroscience*. 2010; 167:232–237. [PubMed: 20149845]
- Schmahmann JD. An emerging concept: The cerebellar contribution to higher function. *Arch Neurol*. 1991; 48:1178–1187. [PubMed: 1953406]
- Schmahmann JD. The cerebrocerebellar system: Anatomic substrates of the cerebellar contribution to cognition and emotion. *Int Rev Psychiatry*. 2001; 13:247–260.
- Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain*. 1998; 121:561–579. [PubMed: 9577385]
- Schwarz C, Schmitz Y. Projection from the cerebellar lateral nucleus to precerebellar nuclei in the mossy fiber pathway is glutamatergic: A study combining anterograde tracing with immunogold labeling in the rat. *J Comp Neurol*. 1997; 381:320–334. [PubMed: 9133571]
- Schmahmann JD, Killiany RJ, Moore TL, DeMong C, MacMore JP, Moss MB. Cerebellar dentate nucleus lesions impair flexibility but not motor function in monkeys. *Soc Neurosci Abstr*. 2004; 34:254–12.
- Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI. Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci*. 1998; 18:2697–2708. [PubMed: 9502827]
- Sivaswamy L, Kumar A, Rajan D, Behen M, Muzik O, Chugani D, Chugani H. A diffusion tensor imaging study of the cerebellar pathways in children with autism spectrum disorder. *J Child Neurol*. 2010; 25:1223–1231. [PubMed: 20179000]
- Snider RS, Maiti A, Snider SR. Cerebellar pathways to ventral midbrain and nigra. *Exp Neurol*. 1976; 53:714–728. [PubMed: 1001395]
- Stoodley CJ. The cerebellum and cognition: Evidence from functional imaging studies. *Cerebellum*. (in press).
- Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation in the brain of patients with autism. *Ann Neurol*. 2005; 57:67–81. [PubMed: 15546155]
- Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ. Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: A stereological experiment using calbindin-D28k. *Cerebellum*. 2008; 7:406–416. [PubMed: 18587625]
- Zuo J, De Jager PL, Takahashi KA, Jiang W, Linden DJ, Heintz N. Neurodegeneration in Lurcher mice caused by mutation in delta2 glutamate receptor gene. *Nature*. 1997; 388:769–773. [PubMed: 9285588]

Fig. 1.

Representative coronal sections illustrating placements (gray shaded areas) of (A) stimulating electrodes in the dentate nucleus (DN), (B) DA recording electrodes in the medial prefrontal cortex (mPFC), (C) infusion cannulae in mediodorsal thalamus (ThN md) and ventrolateral thalamus (ThN vl), and (D) infusion cannulae in the ventral tegmental area (VTA). Numbers correspond to mm from bregma. Dashed line in (B) depicts the lateral to medial angle of recording electrode implantation. All stimulating electrode and infusion cannulae were implanted vertically. Sections were adapted from the mouse atlas of Franklin and Paxinos, (2001).

Fig. 2.

Individual examples of medial prefrontal cortex DA release responses evoked by electrical stimulation (black bar, 100 pulses at 50 Hz) of the dentate nucleus (DN) 3 min before and 3 min after infusions of PBS into the ventral tegmental area (VTA) and kynurenate or lidocaine into the mediodorsal thalamus (ThN md).

Fig. 3.

Average percent decrease in dentate nucleus (DN) stimulation-evoked DA responses following lidocaine, kynurenate, or phosphate buffered saline (PBS, control) infusions into the ventral tegmental area (VTA), mediodorsal thalamus (ThN md), and the ventrolateral thalamus (ThN vl), as well as the summed average percent decrease of each drug infused across sites. Infusions of kynurenate or lidocaine into the VTA, ThN md, and ventrolateral thalamus (ThN vl) of separate groups of mice decreased the DA response by approximately 50% ($n = 15$ and 15), 35% ($n = 15$ and 15), and 15% ($n = 15$ and 15), respectively while phosphate buffered saline (PBS, control) had no significant effect in any group.