

**VERMIS-CINGULATE CORTEX INTERCONNECTIONS: A CEREBRO-  
CEREBELLAR CIRCUIT IN THE RAT**

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University of Pittsburgh, 2013

Classic observations suggest that the cerebellum is involved in affect and its expression. The neural substrate that mediates this behavior has been unclear. Recently, Sacchetti and his colleagues demonstrated that lesions involving portions of lobules V and VI in the rat cerebellar vermis abolish the consolidation of fear conditioning (Sacchetti et al., 2002). To gain some insight into the circuitry that mediates this effect, we used retrograde transneuronal transport of rabies virus (RV) to examine inputs to and outputs from vermal lobule VI. We found that the cingulate cortex (Cg) is the origin of a substantial disynaptic projection to lobule VI. In addition, we found that the fastigial nucleus and portions of vermal lobule VI are the origin of substantial disynaptic and trisynaptic projections to Cg. These observations suggest that a closed-loop circuit exists between vermal lobule VI and Cg. Furthermore, our results raise the possibility that aspects of fear conditioning, in particular and emotion, in general may be influenced by this cerebro-cerebellar circuit.

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## **PREFACE**

I would like to thank Dr. Peter Strick for allowing me the opportunity to learn in his laboratory, and providing generous support and encouragement. I also thank Dr. Isabelle Billig for training, mentorship and academic nurturing during my time at the University of Pittsburgh. I appreciate my husband, Adam Jensen, for standing with me always. This manuscript is dedicated to my father, Dr. John Galgiani.

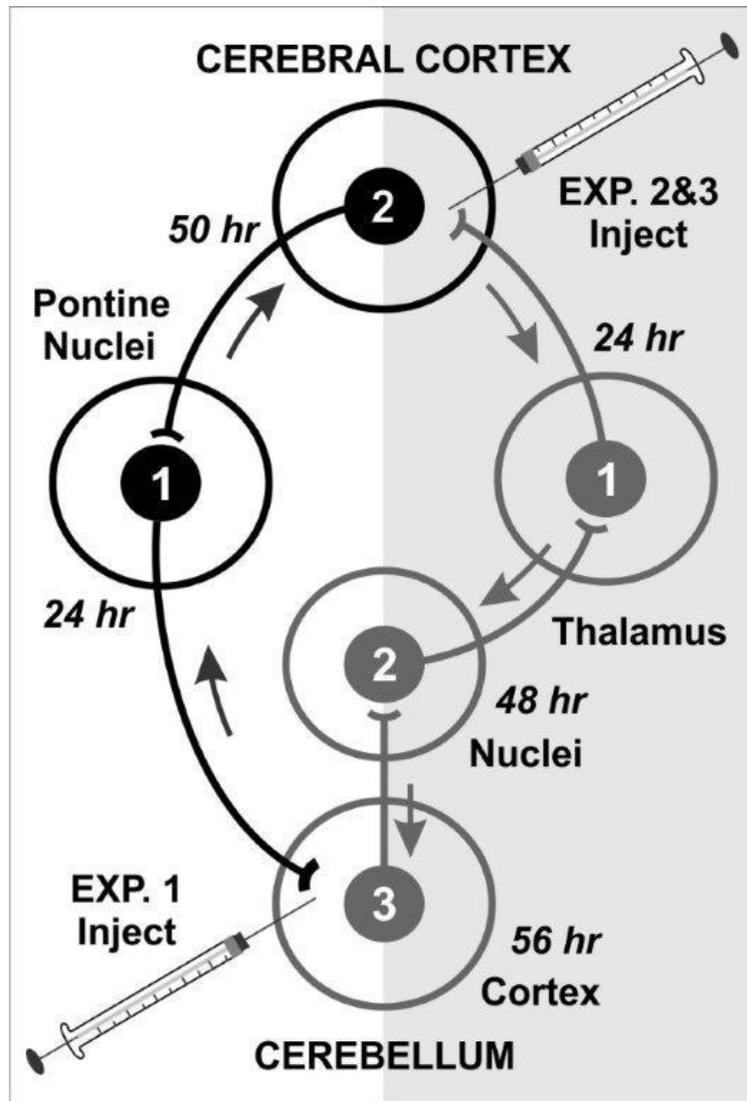
## INTRODUCTION

There is a growing understanding that the cerebellum is involved in aspects of cognition, as well as in motor control (Baillieux et al., 2008; Strick et al., 2009; Watson, 1978). On the other hand, the notion that the cerebellum is involved in affect and its expression remains somewhat controversial. The results of classic studies indicate that lesions of the cerebellum or the deep cerebellar nuclei decrease defensive behaviors and promote docility (Berntson and Schumacher, 1980; Bradley et al., 1987; Chambers and Sprague, 1955; Kling et al., 1979; Peters and Monjan, 1971; Supple et al., 1987). Schmahmann and his colleagues have identified a cognitive-affective syndrome that results from lesions of the posterior lobe of the cerebellum and the vermis (Schmahmann, 1991; Schmahmann, 2004). The affective changes associated with this syndrome included “personality change with blunting of affect or disinhibited and inappropriate behavior” (Schmahmann and Sherman, 1998) These and other results have been the basis for speculation about cerebellar participation in the processing of emotion. However, a clear anatomical substrate to support cerebellar involvement in this aspect of behavior has been lacking.

Recently, Sacchetti and his colleagues provided evidence that specific regions of the rat posterior vermis (portions of lobules V and VI) are critical for the consolidation of fear conditioning (Sacchetti et al., 2002). In addition, they found that the parallel fiber synapses onto Purkinje cells in vermal lobules V and VI display long-term post-synaptic changes associated with fear conditioning (Sacchetti et al., 2004; Zhu et al., 2007). By identifying a specific site in

the cerebellum, Sacchetti and his colleagues have provided us with an opportunity to explore the neural substrate underlying the cerebellar involvement in fear conditioning. In essence, we have used retrograde transneuronal transport of rabies virus to examine the multi-synaptic inputs to and outputs from portions of vermal lobule VI.

We performed a series of three experiments. In the first experiments, we injected RV into vermal lobule VI of the rat and used retrograde transneuronal transport of the virus to define the di-synaptic inputs to the injection site. We found that the cingulate cortex (Cg) is the origin of a major disynaptic projection to vermal lobule VI. In the second experiments, we injected RV into Cg of the rat and used retrograde transneuronal transport of the virus to define the di-synaptic inputs from the deep cerebellar nuclei (DCN) to this injection site. We found that the fastigial nucleus is the origin of a major di-synaptic projection to Cg. In the third experiments, we injected RV into Cg of the rat and extended the survival time to define the tri-synaptic inputs from the cerebellar cortex to the injection site. We found that portions of vermal lobule VI are the origin of a major tri-synaptic projection to Cg. These observations suggest that a closed-loop circuit exists between vermal lobule VI and Cg. Furthermore, a region of the posterior vermis both receives from and projects to Cg, a cortical region thought to be involved in fear and affect. (Etkin et al., 2011). These results raise the possibility that aspects of fear conditioning, in particular, and emotion, in general, may be mediated by a cerebro-cerebellar circuit between the posterior vermis and Cg.



**Figure 1.** Experimental Schematic

In experiment 1, RV is transported retrogradely 2 synapses, from the cerebellum, to the cerebral cortex. In Experiments 2&3, RV is transported retrogradely either 2 or three synapses, to the DCN or to the cerebellar cortex.

## 1.0 MATERIALS AND METHODS

This report is based on observations from 9 rats (Male, *Sprague-dawley*) in a series of three experiments. First, RV was injected into lobule VIc of the cerebellar vermis for transport to the cerebral cortex (n=3). In the second series RV was injected into the Cg for transport to DCN (n=3). In the third series RV was injected into the Cg for transport to the cerebellar cortex (n=3). The procedures for each series are similar, differing mainly in injection location, volume of injectate and survival time (Figure 1).

We complied with guidelines from the Association for the Assessment and Accreditation of Laboratory Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by both the Institutional Animal Care and Use Committee and the Biosafety Committee. Biosafety practices conformed to Biosafety Level 2 regulations as outlined in Biosafety in Microbiological and Biomedical Laboratories (Department of Health and Human Services publication No. 93-8395). Procedural details for handling virus and virus-infected animals have been published previously (Kelly and Strick, 2000).

## **1.1 SURGICAL INJECTIONS**

Each rat was given a preoperative dose of analgesic (Buprenorphine, 0.1mg/kg). Surgeries were performed under general anesthesia (ketamine 75mg/kg, Xylazine 5mg/kg) and aseptic conditions. Rats were placed in a stereotactic frame (David Kopf Instruments). The skin above the skull was retracted and the brain was accessed through a small hole made with a dental drill (Dremel) or a rongeur. We used a glass-tipped microsyringe (10µl, Hamilton) held by a micromanipulator to inject a mixture of rabies virus (RV; N2C strain) and cholera toxin, beta subunit (CTb, List Biological, 0.02%) slowly into the brain. The needle was held in place for 7 minutes before being gently withdrawn. For cerebellar injections, the needle was visually placed into lobule VIc of the vermis and a single injection of 0.4-0.5µl was made 0.6mm below the surface. For cerebral cortex injections, defined stereotactic coordinates (+2.7 mm AP, 0.5mm ML, 1.9 mm DV) were used to place injections and a total volume of 0.4-0.5µl was injected over 2-3 depths. After the needle was removed, the brain was rinsed with sterile saline, the burr hole was filled with Gelfoam, and the animal was sutured and returned to its home cage to recover. Post-operative analgesia (buprenorphine, 0.1mg/kg) was given 8-12 hours after surgery.

## **1.2 PERFUSION AND HISTOLOGY**

The timing of perfusion was important to allow transport of RV to the desired number of neuronal orders. Previous experiments had allowed us to determine the correct timing for each series of experiments. Cerebellar injected rats survived 50 hours, and cerebral cortical injected animals survived either 48 hours (to allow second-order transport of RV to deep cerebellar

nuclei) or 56 hours (for third order transport to cerebellar Purkinje cells). Animals were deeply anesthetized with sodium pentobarbital (100 mg/kg) and transcardially perfused using a sequence of three solutions (Phosphate buffer 1M, formalin, and a formalin/10% glycerin solution). Brains were removed and allowed to post-fix for 24h in a chilled (4°C) formalin/20% glycerin solution. Serial sections 50 µm thick were cut on a freezing microtome in either the coronal or sagittal plane. Half were processed as free-floating sections to illuminate either RV or CTb labeling. One out of every ten sections was stained with cresyl violet for the analysis of cytoarchitecture. We used the avidin-biotin peroxidase method (Vectastain, Vector Laboratories). The RV processing used a monoclonal antibody (M957; supplied by A. Wandeler-Animal Disease Research Institute, Nepean, 1:20,000) that is specific for the P antigen expressed by RV. CTb labeling was revealed using a commercially available goat polyclonal antiserum (1:10,000) and an affinity-purified secondary antibody (donkey anti-goat IgG, Jackson Laboratories) along with the peroxidase procedure for RV. After processing, sections were mounted onto gelatin-subbed slides and allowed to dry before being coverslipped with Cytoseal.

### **1.3 ANALYTIC PROCEDURES**

We examined at least every fourth section under a light microscope, using brightfield and darkfield illumination. Sections were drawn using a computer-based plotting system which captures the movement of the microscope stage as x-y coordinates (MD2, Accustage). We recorded the location of labeled neurons and drew tissue outlines along with important cytoarchitectonic landmarks. These data were then transferred to custom software.

### **1.3.1 Injection sites**

Because RV does not label the site of injection well, CTb immunoreacted sections were used to determine the spread of each RV injection. Additionally, for cerebellar injections, we used first order data from the inferior olive to identify the olivo-cortico-nuclear zone which corresponded to each injection and determine whether the injection had crossed the cerebellar midline(Buisseret-Delmas and Angaut, 1993; Suzuki et al., 2012).

### **1.3.2 Unfolded Maps**

Custom software was used to create unfolded maps of the cerebral cortex and cerebellum. This method has been previously described (Dum and Strick, 1991). Briefly, sections were aligned at a landmark (either the dorsomedial pole of the cortex, or the fissure between lobule VI and VII of the cerebellum). The “unfolding line” was made either around layer V (cerebral cortex) or around the cerebellar surface at the border between the molecular layer and granular cell layer (cerebellum). Cerebral maps were standardized by aligning landmarks to an unfolded map generated from atlas sections (Watson and Paxinos, 1998). We then divided these maps into 200µm square bins and created density maps to determine the regions that were most strongly labeled. For cerebellar maps, most labeling was found at the lobular surface, so “surface” maps were created by extending the unfolding line across lobules instead of following the contours into the fissures (Kelly and Strick, 2003).

## 2.0 RESULTS

In our first series of injections into lobule VIc of the vermis, we found that a number of cortical areas project to a localized region of the rat cerebellum. The medial wall of the hemisphere had the highest density of cells immunopositive for rabies virus. The most substantial projection to lobule VIc of the cerebellar vermis was from the cingulate cortex (Cg). Consequently, in our next series of injections, we targeted this Cg “hot-spot” for cortical injections and found labeling in all three deep cerebellar nuclei (DCN), including the fastigial nucleus. In our third series of injections we again targeted the Cg and allowed RV to travel three synapses to cerebellar Purkinje cells. We found that the most densely labeled region of the cerebellum included lobule VIc of the vermis. Therefore, lobule VI and Cg appear to be connected in a closed-loop cortico-cerebellar network.

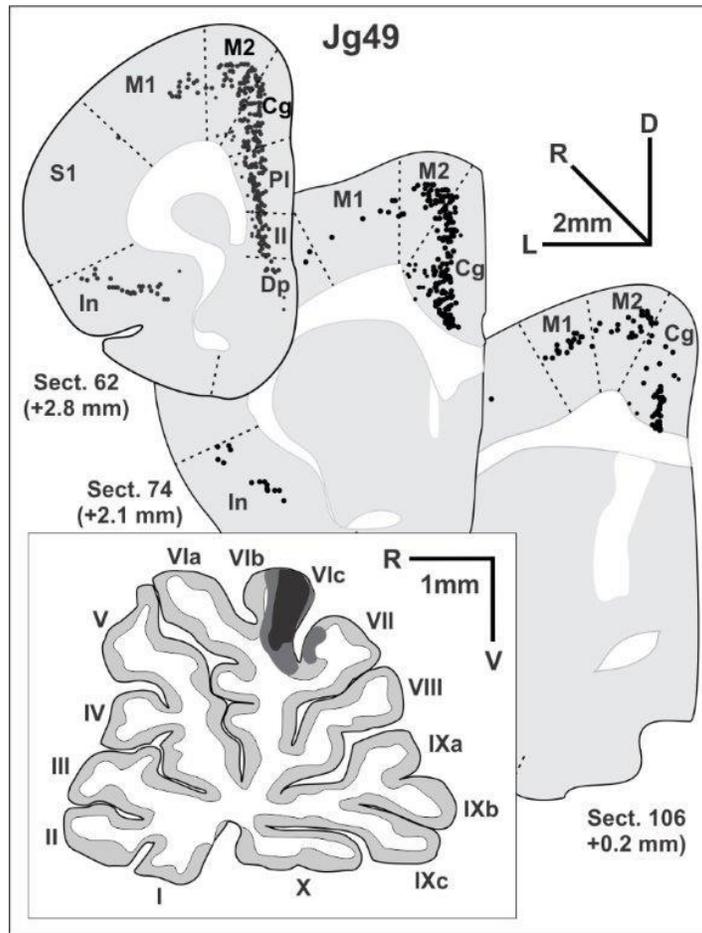
### 2.1 CEREBELLAR INJECTIONS

We mapped the location of CTb-staining in the cerebellar cortex and examined the first-order labeled cells in the inferior olive to determine the spread of each cerebellar injection. Figure 2 shows a representative injection site into lobule VIc of the cerebellar vermis. The two shades of grey indicate the primary (dark) and secondary (light) zones of injection, judged by the strength of CTb staining. Only the Caudal Medial Accessory olive (subunit C) was labeled for all three

cases, indicating that all injections were confined to “zone A” of vermal lobule VIc. One injection crossed the midline (JG49). Additionally, we confirmed the presence CTb labeled cells in the caudal pontine nuclei. Prior studies indicate that the pontine nuclei are a major source of cortical input to the cerebellum (Glickstein and Doron, 2008; Legg et al., 1989; Schmammann and Pandya, 1997). Based on this, we consider that the pontine nuclei represent the most likely mono-synaptic link to the RV labeled neurons seen in the cerebral cortex.

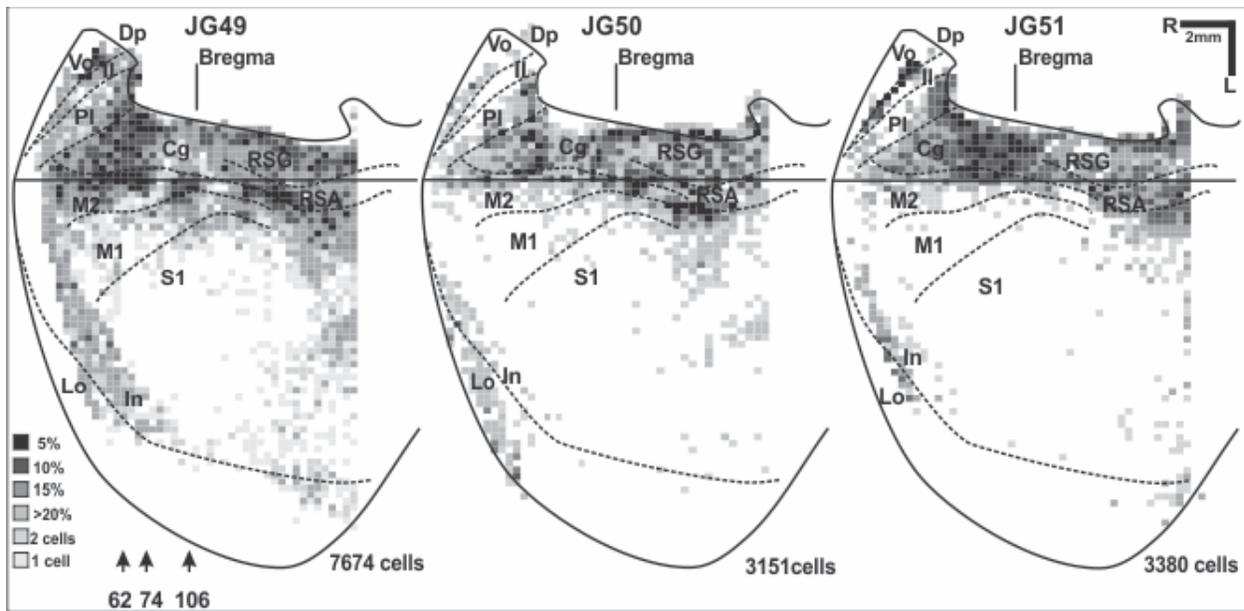
At survival times of 50 hours we observed second-order transport of the rabies virus to layer V pyramidal cells in the cerebral cortex. We also examined the amygdala, periaqueductal grey (PAG) and hypothalamus for labeled cells. There was no evidence of first or second order labeling in the amygdala. We located some labeling of second-order RV cells in the hypothalamus. These were sparse but widely distributed through out the anterior-posterior spread of the hypothalamus. We also found a small population of second order cells in the dorsolateral PAG. The details of the cortical labeling will be the focus of the present report.

Figure 2 shows coronal slices of the contralateral cerebral cortex at selected intervals, and Figure 3 shows an unfolded cortical map of the contralateral cortex for each case. All animals in this report survived 50 hours, as shorter survival times in pilot experiments were not effective in allowing the virus to progress from the cerebellum to the cerebral cortex. At 50 hours, only one case exhibited “infra/supra” layer V label (JG49, 3% of total labeling). Most of the labeled cells are located in the medial wall of the hemisphere. Both cerebral hemispheres in each case have approximately the same number of cells. Cg is the most densely labeled region (30%), while there is less labeling in M1 (5%). Other regions of dense label in the medial wall of the cortical hemisphere include retrosplenial cortex (17%), secondary motor cortex (13%) orbital cortex (12%) and prelimbic cortex (10%).



**Figure 2:** JG49 Cerebellar injection and cortical labeling.

Unfolded cortical density maps of RV labeling after cerebellar vermis injections show the distribution of labeled cells (fig. 3). Black squares represent bins with the highest density of labeled neurons. These are distributed in more than one area, demonstrating that several cortical regions converge onto lobule VIc area in the vermis. However, Cg was the most densely labeled region. All three maps were standardized and used to create a composite map (fig. 6).

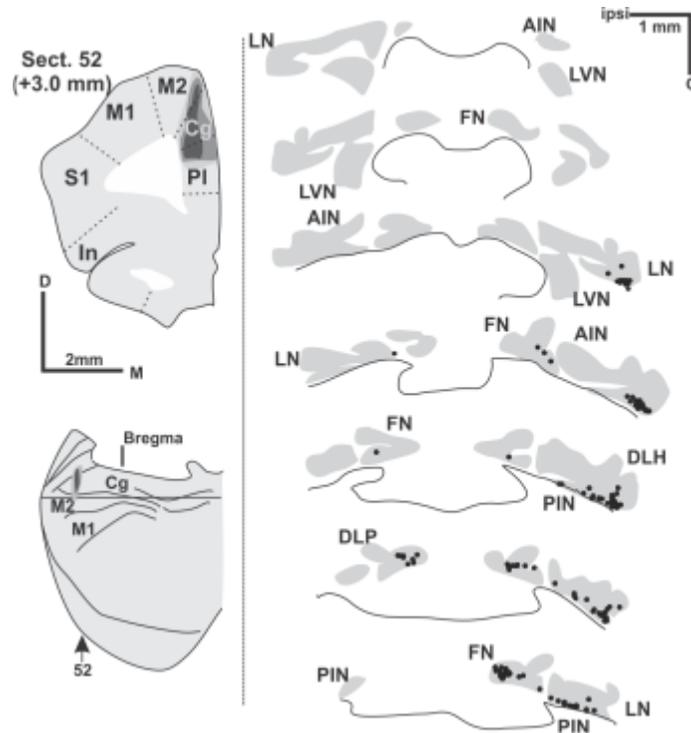


**Figure 3:** Unfolded cortical density maps; RV labeling in the cerebral cortex. Bins (200µ square) are sorted from most dense (black) to least dense (light grey).

## 2.2 CINGULATE CORTEX INJECTIONS

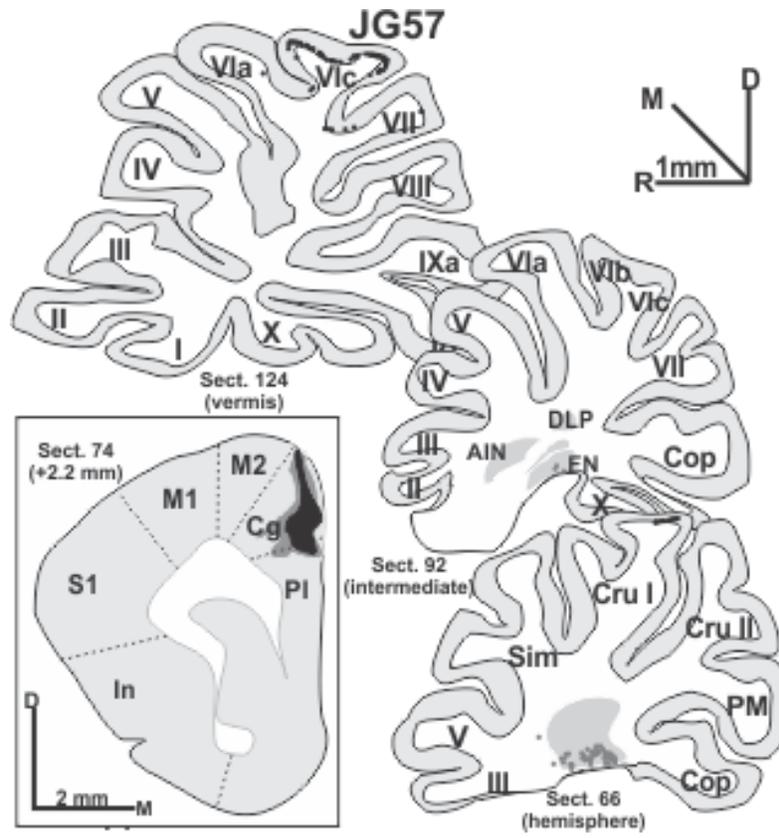
We targeted Cg for injections of RV in the second and third series of experiments to determine if the same area of the cerebellar vermis sent input to Cg as received input from Cg. Animals in the second series of injections survived 48 hours to visualize RV transport to the deep cerebellar nuclei, and animals in the third series survived 56 hours to visualize RV transport to cerebellar Purkinje cells. After reconstructing each injection site, we determined that all injections covered at least Cg (+3.7 mm- +2.5 mm AP) with some spread into the dorsal pre-limbic cortex (fig. 4 and 5). There was negligible spread into secondary motor cortex. We observed first order labeling in the thalamus, and the topography of this projection has been reported elsewhere (Hoover and Vertes, 2007).

RV was transported exactly two orders in the first set of cortical injections. We verified this based on the presence of labeling in the DCN and its absence in Purkinje cells. We found cells in the ventral portions of the lateral and the posterior interpositus nuclei, and in the caudal-central portion of the fastigial nucleus. An example of an injection site into Cg, with resulting labeling in the DCN is shown in figure 4 (sections every 200  $\mu\text{m}$ ). Thus, portions of all three deep cerebellar nuclei, including the fastigial nucleus, send convergent output to Cg.

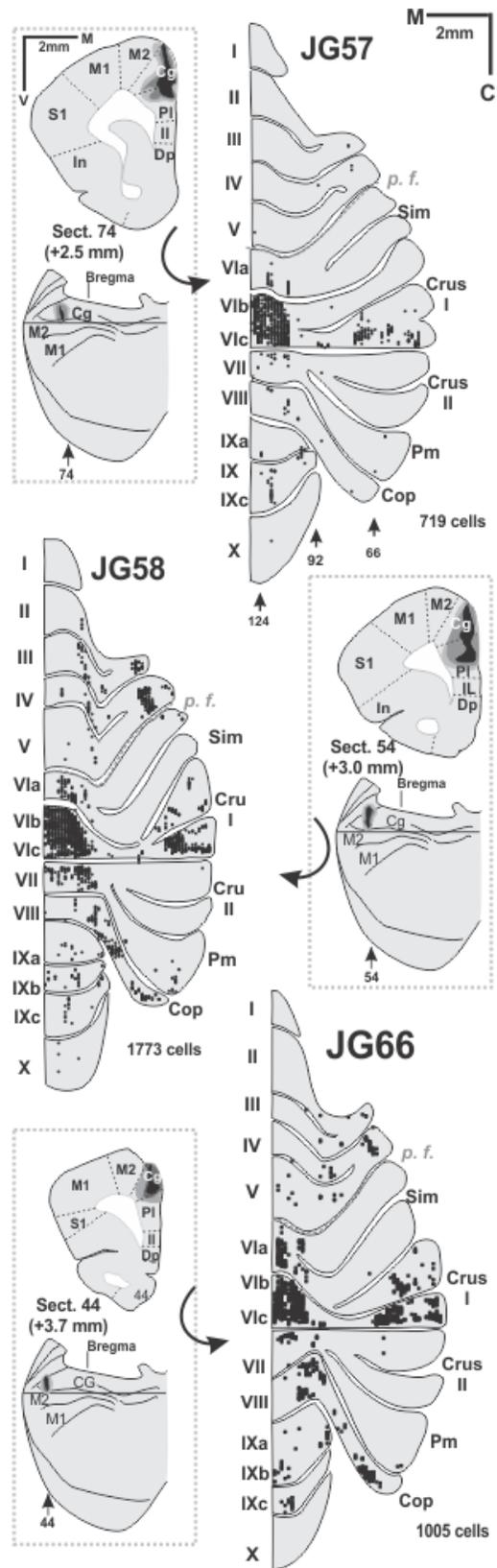


**Figure 4:** Labeling in DCN after RV injection into Cg

In the third series of experiments we allowed tri-synaptic transport of RV to cerebellar Purkinje cells (fig. 5 and 6). There were no labeled granule cells or inferior olive cells at this survival time. The predominant feature was dense labeling in lobule VI of the cerebellar vermis (45% of total labeling in a hemisphere). This region overlaps with VIc of zone A, the same area that was injected during the first series of cerebellar experiments. Labeling also appeared in small patches mainly at the surface of hemispheric lobules III, V, VIII and in Crus I. Figure 6 demonstrates this on an unfolded cerebellar hemisphere, contralateral to the injection site in CG. This result suggests that the cingulate cortex and lobule VIc of the cerebellar vermis share closed loop architecture via the fastigial nucleus. To help visualize the overlap between the separate components of this experiment, we created composite maps that standardized and aligned all three cerebellar injections, and all three cingulate cases. The results are shown in figures 5 and 6.



**Figure 5:** RV injection into Cg with cerebellar labeling



**Figure 6: Unfolded cerebellar maps**

### **3.0 DISCUSSION**

We selected a specific site in the cerebellar vermis and examined the input to and output from it based on observations that lobules V and VI participate in fear conditioning consolidation. The posterior vermis, in particular, has been suggested to be the region that mediates affective behavior in the cerebellum. We therefore sought to determine the neural substrate that might underlie these behavior attributes. We found no evidence of a direct or di-synaptic projection from the amygdala to lobule VI of the cerebellar vermis. The amygdala is thought to be critical in the acquisition of a conditioned fear response (Kim and Jung, 2006; LeDoux, 2000). Lack of input from the amygdala to lobule VI of the vermis suggests that the input from other brain regions mediates a cerebellar role in fear, or that amygdala plays a less direct role.

#### **3.1 INPUT TO VIC OF THE CEREBELLAR VERMIS**

We found that neurons in the hypothalamus and the ventrolateral PAG projected to our injection sites into lobule VIc. Both of these regions may play a role in the expression of autonomic behaviors that are part of a fear response (Brandao et al., 2008; Johnson and Shekhar, 2012). Indeed, it has previously been reported that mono-synaptic connections between the hypothalamus and the cerebellum exist (Haines et al., 1997; Onat and Cavdar, 2003). The contribution from hypothalamo-cerebello connections has also been suggested to be involved in

the cardiac effects seen by midline or fastigial cerebellar manipulations (Supple et al., 1988; Supple, 1993). Thus, communication between the hypothalamus and the cerebellum may contribute to the role lobule VI plays in fear expression (Zhu et al., 2006).

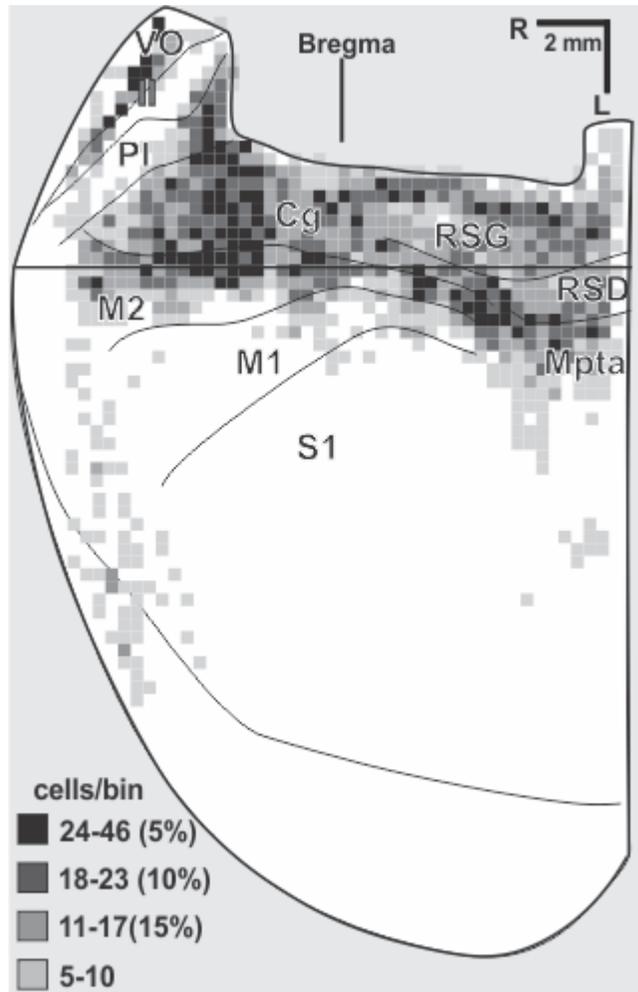
On the other hand, lobule VI of the cerebellar vermis in rats has been shown to play a role in fear conditioning consolidation, and not in the expression of fear (Sacchetti et al. 2002). Interestingly, we found that several areas of the cerebral cortex project to lobule VIc of the vermis. Convergence to the cerebellum has been recently reported by others who have used transneuronal tracers to map the di-synaptic connections from the cerebral cortex to the cerebellar vermis (Coffman et al., 2011; Suzuki et al., 2012). We noted that the majority of input to lobule VIc of the vermis does not come from primary or secondary motor cortex, implying that cortical information to lobule VI is not directing voluntary movement of the extremities. This is consistent with classical interpretations of cerebro-cerebellar somatotopy (Snider and Eldred, 1952).

Instead, input comes from orbital cortex, retrosplenial cortex, and medial pre-frontal cortex, including Cg. These cortical areas are known to be involved with higher functions of learning and memory (McAlonan and Brown, 2003; Nieuwenhuis and Takashima, 2011; Robinson et al., 2011). One possibility is that each of these areas plays a part in the fear behaviors attributed to lobule VI of the cerebellum, and that their convergent input is important in fear consolidation.

We found that the densest source of input to vermal VIc was from Cg. The rodent Cg, like the primate Cg, is most likely heterogeneous in function. Both motor and non-motor territory have been identified in the rat (Wang et al., 2008). However, the boundaries are not well delineated. Regions of the Cg that, when stimulated, evoke nose, eye and whisker movement

(Brecht et al., 2004) overlap with regions that have also been demonstrated to be involved with fear learning and pain (Johansen et al., 2001). This apparent thrift in the use of cortical space can make it a challenge to infer function from location in the rat brain. However, there is evidence to suggest that rostral Cg is more likely to mediate non-motor, affective behaviors such as fear learning (Malin et al., 2007), and caudal Cg is more likely to mediate limb movement (Wang et al., 2008). Therefore, despite evidence that Cg may participate in other behaviors, it is reasonable to say that input to the cerebellar vermis from Cg could enable lobule VI to participate in fear consolidation.

The role Cg plays in fear learning was recently reviewed by two groups who found that evidence supports a role for Cg in the acquisition of fear (Courtin et al., 2013; Einarsson and Nader, 2012). Pre-training blockades or lesions of the Cg attenuate the learning of fear conditioning or inhibitory avoidance (Bissiere et al., 2008; Johansen and Fields, 2004; Tang et al., 2005; Zhao et al., 2005). Early immediate gene expression in Cg is correlated with fear learning retrieval (Beck and Fibiger, 1995; Frankland et al., 2004; Smith et al., 1992; Tulogdi et al., 2012). This has been interpreted to mean that Cg is active in modifying and maintaining long term aversive memories. Furthermore, fear conditioning is correlated with an increase in dendritic spine growth and interfering with dendritic spine growth or kinase activity after training affects fear learning (Holahan and Routtenberg, 2007; Restivo et al., 2009; Vetere et al., 2011)



**Figure 7:** Composite cortical map (n=3)

Cg input to lobule VIc of the vermis, therefore, has the potential to provide the cerebellum with higher order information about fear conditioning, providing a route by which this region of the cerebellum may participate in fear learning. Though the amygdala does not appear to be directly linked to lobule VI of the vermis, it could interact with the cerebellum indirectly via Cg. Cg and the amygdala are anatomically connected (Gabbott et al., 2005; Hoover and Vertes, 2007) and evidence supports a functional relationship as well. Infusion of a cholinergic agonist into Cg improves learning of inhibitory avoidance, however, a chemical lesion of the basolateral amygdala prevents this enhancement (Malin 2006). Interestingly, a blockade of the amygdala can also prevent cerebellar synaptic changes induced by fear conditioning (Zhu et al., 2011).

### **3.2 OUTPUT FROM VI OF THE VERMIS**

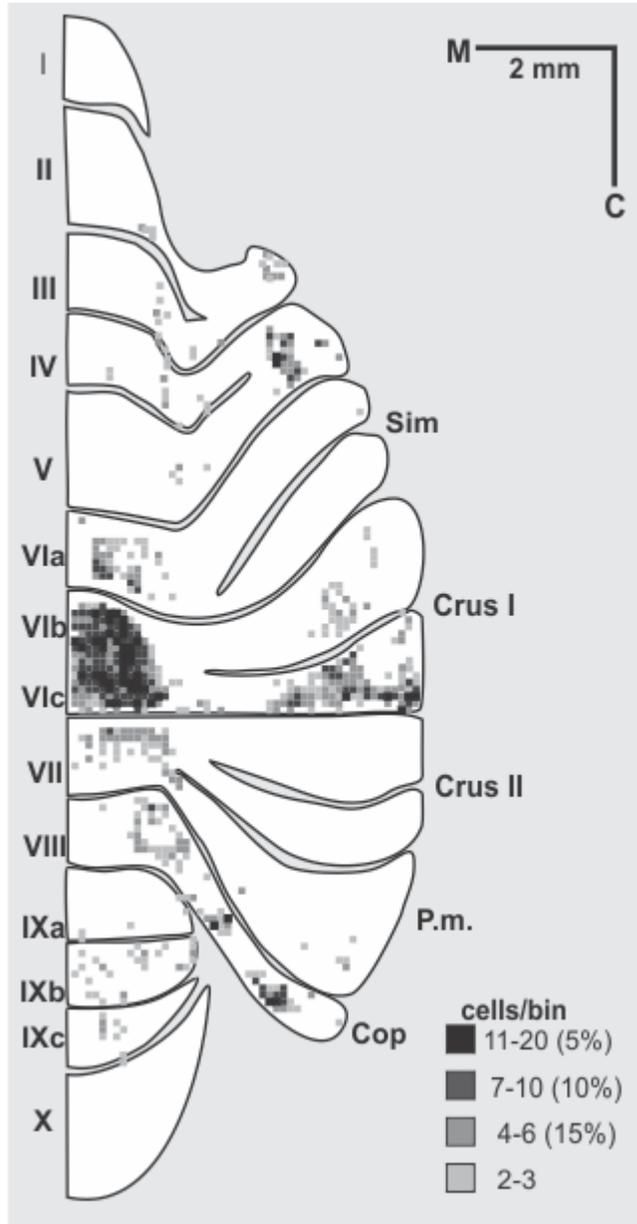
We also examined whether Cg was the target of cerebellar output. Di-synaptic transport of RV from Cg labeled neurons in the lateral, interpositus and fastigial DCN. Specifically, cells were found in the ventral portions of lateral and interpositus nuclei, and in the caudal fastigial nucleus. These regions may represent areas of non-motor output in the rat DCN. In the primate, cerebellar output to different cortical regions is segregated into distinct output channels (Middleton and Strick, 2001). Additionally, there is a dorsal/ventral distinction for motor versus non-motor output from the interpositus (Lu et al., 2012). The precise topography of output has not been determined in the rat. However, the fastigial nucleus has been proposed as the source of affective cerebellar output (Stoodley and Schmammann, 2010). Also, the labeling we see in the lateral and the posterior interpositus nuclei after injecting in the rostral Cg parallels the finding of ventral

labeling in these nuclei after injecting into non-motor primate cortex. In this way, it is interesting that the output we observe from the rat DCN to Cg shares some features with the non-motor output channels of the primate.

In a third and final series of experiments, we allowed RV injections to transport from Cg through three synapses to cerebellar Purkinje cells. We found that, to some degree, cerebellar cortical output to Cg is also convergent as labeling appears in both medial and lateral regions of the cerebellum. However, the most substantial proportion of labeled cells was found in lobule VI of the vermis. Importantly, this region overlaps with the area of the cerebellar vermis that receives input from Cg. Thus, our data imply that a closed loop exists between lobule VI of the cerebellar vermis and Cg.

### **3.3 A CINGULATE-CEREBELLAR LOOP**

It is notable that these two regions exhibit closed-loop macroarchitecture. Motor and non-motor regions of the cerebral cortex have been shown to communicate with the cerebellum by way of separate closed cerebro-cerebellar circuits (Kelly and Strick, 2003). In essence, these loops provide the anatomic means for the cerebellum to participate in both movement and cognition. Indeed, closed-loop architecture may describe the fundamental unit of interactions between the cerebellum and the cerebral cortex (Bostan et al., 2013). In this system, Cg, a cortical region involved in the acquisition of fear, sends information to a specific region of the cerebellum, lobule VIc. Significantly, this region of the cerebellum, which may be important for fear consolidation, also influences Cg. Thus, this unique circuit between lobule VIc of the vermis and Cg provides a route by which the cerebellum could participate in an affective behavior like fear.



**Figure 8:** Composite cerebellar map (n=3)

The notion that the cerebellum is involved in affective behaviors and cognition is not without controversy, and some argue that links with higher order brain centers in fact function to control eye movement and are only minimally involved in cognition (Glickstein and Doron, 2008). Indeed, the medial cerebellum including the fastigial nucleus and lobules VIc and VII are termed the oculomotor vermis for their involvement in eye movement (Thier et al., 2002). At the same time, our cerebellar injections into VIc of the vermis label regions of the cerebral cortex where others have shown have eye-movement properties. Stimulation in a region of cortex similar to our area of high density labeling in Cg may elicit lid or periorbital movement (Brecht et al., 2004), and we show labeling in a region that overlaps with the rat “frontal eye field”(FEF) (Guandalini, 2001). An alternative interpretation for our data is that the cerebro-cerebellar connections we see have oculomotor functions.

These two interpretations, however, may not be mutually exclusive. Rats are afoveate, and their FEF receives information from parietal and whisking-associated cortical areas. (Guandalini, 1998). Thus, the rat FEF is additionally implicated in exploring and orienting to the environment. The rostral Cg has also been shown to control pupil size (Guandalini, 2003) suggesting an autonomic role. Therefore, eye movement-related activity does not preclude these areas from cognitive or affective involvement. Indeed, a hallmark feature of schizophrenia or bipolar disorder in humans is an inability to make smooth pursuit eye movements (Martin et al., 2011). Smooth pursuit movement is, after all, a volitional process, which requires cognition, target selection and attention (Barnes, 2008). Therefore, it would not be surprising if there were overlap between cortical regions associated with orienting to relevant, potentially threatening stimuli and acquiring fear memories.

A “limbic loop” linking the cerebellum to temporal and frontal regions of the cortex has been proposed before (Heath and Harper, 1974; Snider and Maiti, 1976) based on physiological data, though an anatomic path has not been previously reported. Clinical evidence has led to the hypothesis that the vermis, lobule VI in particular, is likely to be involved in emotion (Stoodley and Schmahmann, 2010; Timmann et al., 2010). There are alterations in both the cerebellar vermis and in prefrontal brain matter in certain psychological disorders such as autism, obsessive compulsive disorder and eating disorders (Amianto et al., 2013; Milad et al., 2013; Noriuchi et al., 2010) Recently, vermis-cingulate interactions have been specifically indicated in an imaging study of depression (Alalade et al., 2011). Our data suggest that Cg and lobule VI of the vermis are interconnected, and such a relationship may explain some of these affective behaviors. In the rat, vermal-prefrontal circuitry has the potential to mediate fear learning. Further study of the interconnection between the vermis and the cerebral cortex will not only help to increase our understanding of fear and anxiety, but may also contribute new insights to other psychological disorders.

## **LIST OF ABBREVIATIONS**

Cg: Cingulate Cortex

Cop: Copula Pyramidis

Cru I: Crusiform lobule I

Crus II: Crusiform lobule II

DCN: Deep Cerebellar Nuclei

Dlp: Dorsal Peduncular Cortex

Il: Infralimbic Cortex

In: Insular Cortex

Lo: Lateral Orbital Cortex

M1: Primary Motor Cortex

M2: Secondary Motor Cortex

Pm.: Paramedian lobule

Pl: Pre-limbic Cortex

RV: Rabies Virus

Sim: Simple lobule

Vo: Ventral Orbital Cortex

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