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SPECIAL ISSUE EDITORIAL
Thalamic branches of corticofugal axons from view of a critical eye and great mentor, Ray Guillery

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Ray (Rainner) Guillery was a perfect scientist role model for the young generation. He was very generous in sharing his scientific knowledge and had an enormous sense of humour which showed his brilliant sharp intelligence. We had the privilege to work with him for 4 years (2006–2010) in Marmara University, Istanbul and kept in contact until he passed away in 2017. As neuroscientists, we were not the only ones to greatly benefit from his experience during his stay in the Departments of Anatomy and Pharmacology, he was also in contact with the other departments (Public Health, Histology, Internal Medicine, etc.) of the medical faculty.

Ray came to Istanbul and joined us in 2006 as an emeritus professor in the Department of Anatomy. We felt very fortunate to meet him and study with him in our university because the major research field of the departments of basic sciences of medical faculty is neuroscience and we were aware of the significance of Ray's work in the field. He impressed us not only with his scientific background, extensive knowledge and approach to neuroscience but also with his plain and modest remarks on life. In an interview published in 2008 (Guillery, 2008), he replied to the question about the reason of moving to Turkey and living in Istanbul with these sentences; One of the attractions of my move to Istanbul was the fact that Marmara University welcomed me and offered me an opportunity to continue with some academic activity even though I am well past retirement age, and unfundable for research. Furthermore, he referred to the effect of meeting colleagues in Marmara University on his decision to move to Istanbul in unpublished writings entitled, "Some thoughts about ageing in Istanbul" with these sentences. I had published papers on the thalamic reticular nucleus (some relying to a significant extent on John's (John W. Crabtree) studies) and wrote to Safiye, introducing myself and suggesting that on my next visit to Istanbul I might visit her department. She sent back an enthusiastic positive reply and the next time I was in Istanbul I arranged to visit the people in the Anatomy Department. They were very welcoming, arranged for me to give an informal talk to a small group from the anatomy and pharmacology departments, and encouraged me to visit, to meet students and discuss problems of shared interest. That visit played an important part in helping me decide that when the time came to move from Madison, it might be a good idea to move to Istanbul. I could be close to family, and at the same time keep some academic interests alive in a way that would be far more difficult in the UK or the US. I found wonderful colleagues with an interest in the thalamus and the thalamic reticular nucleus.

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The foremost and very important support that Ray gave to the scientific and academic research activity of the medical faculty of Marmara University was in the preparation of our manuscripts. He made significant contributions to the editing of our manuscripts. Criticism of our papers with a prominent neuroscientist was a precious opportunity and a very challenging activity for us. Ray also appreciated the attention of faculty members and enjoyed studying the manuscripts with his Turkish colleagues. He wrote about writing and doing science in Istanbul in his unpublished writings in the following way: When I first moved here in the summer of 2006, I was given a very nice office with a view of Florence Nightingale museum in Selimiye Barracks. Selimiye Barracks housed a hospital that Florence Nightingale used, and it now houses a Florence Nightingale Museum that I have been meaning to visit for some time. I have placed the desk in my office so that I can see one of these huge historic Florence Nightingale towers when I sit at my desk. It gives me a sense of being in a different environment. I have spent a fair amount of time here working with colleagues on manuscripts they are preparing for English language journals. I enjoy doing this. I have done it for colleagues in Hong Kong and Mainland China for several years, and when the European Journal of Neuroscience was started in the 1980s one of the aims was to help non-native speakers with the structure of their English. The pharmacologists here have involved me in editing a number of manuscripts. I enjoy this greatly. They are (almost) all attractive young women who are all, or at least seem, keen to learn. Four of the students were at a recent epilepsy meeting in Antalya and were spoken of as the "Marmara Angels." We (me and some of the angels) sit down in the pharmacology department with a projector that throws the draft text onto the wall, and then discuss the details. Usually three or four people are involved, and there is an active interchange. Turkish coffee usually accompanies each session, often with some pastries or chocolates as well.

Ray gave many lectures and seminars in the medical faculty of Marmara to different aged academic career groups. He was willing and was happy being with Turkish medical students as well as young research assistants and professors from various departments of the faculty. He liked talking about and discussing science, about the doctrines of life or the origin and improvement of neuroscience in the world, within the historical halls of the old Ottoman building. The description of this atmosphere in his own words is as follows: The medical school (Tip Fakultesi) is in a wonderful old, late Ottoman building with impressive towers and a huge ceremonial entrance that is not used except, I suspect, for special occasions. It encloses a large courtyard, is separated from the road by a narrow garden area, and has significant grounds at the back facing the sea.

It has a sense of space I still admire whenever I go there, usually several times each week, and each time the sense of space impresses me. Today I gave a lecture here in the Marmara medical school building in a wonderful lecture hall with galleries on all four sides, beautifully decorated. A bit reminiscent of the Sheldonian in Oxford. Even the metal staircase leading up to the theatre was in a fine state of repair and the theatre itself had recently been completely redone. The building still surprises me with its hugely ambitious and very beautiful proportions.

In addition to faculty members, he gave noteworthy and fascinating talks to the local neuroscience society (Brain Research Association "BAD," the local neuroscience society of Turkey) and at international Turkish scientific meetings as an invited speaker, in different parts of Turkey including Safranbolu, Antalya and Pamukkale. He was delighted at visiting interesting parts of Turkey, meeting new colleagues and making new collaborations. We thus greatly benefitted from his encouragement and contribution to the progress of neuroscience in Turkey.

Ray also greatly enjoyed hosting visitors from abroad including Chris Walsh, Suzanne Tarlow, YaYun Wang, Murray and Marge Sherman and Didier Pinault. He shared his feelings about these visits with these words: One of the advantages of living in a city as beautiful as Istanbul is that it should make friends from other places want to come and visit. I have a small apartment, in the plans for which I included a spare bedroom with a large double bed because I wanted to be able to entertain visitors. Chris Walsh came through at an early stage, and gave a lecture at Marmara hospital to a large audience of clinicians who were interested in the development of the cerebral cortex, or else were under orders to attend the lecture. It was good to have him visit this side. Another visitor who came through was a US neuroscientist, Suzanne Tarlov to talk to me about albinism. Another visitor came for a brief weekend in Istanbul during a postdoctoral IBRO fellowship that she had been awarded to study in Paris for 1 year. This was YaYun Wang, an anatomist from Xi'an who had several years earlier attended a course in Hong Kong that I helped teach and who had then, a few years later, when she was a PhD student, been a guide to the city in Xi'an for Hector Chan and me when we visited there. We had a visit from Didier Pinault, French neurophysiologist from Strasbourg. For me it was a great opportunity to have him visit, discuss some issues with him and have him meet with Safiye and Filiz and other Turkish colleagues here. I am looking forward to visit next week from Murray and Marge Sherman. They will stay with me on the Saturday and Sunday. I have been trying to think of good touristy things to do with them, but will have to wait till they get here to find out what they really want to do. For me it should be a chance to see the two Shermans again.

Ray had enormous experience of the anatomy, cellular structure and, in particular, the function of the thalamus and cortex. He wrote

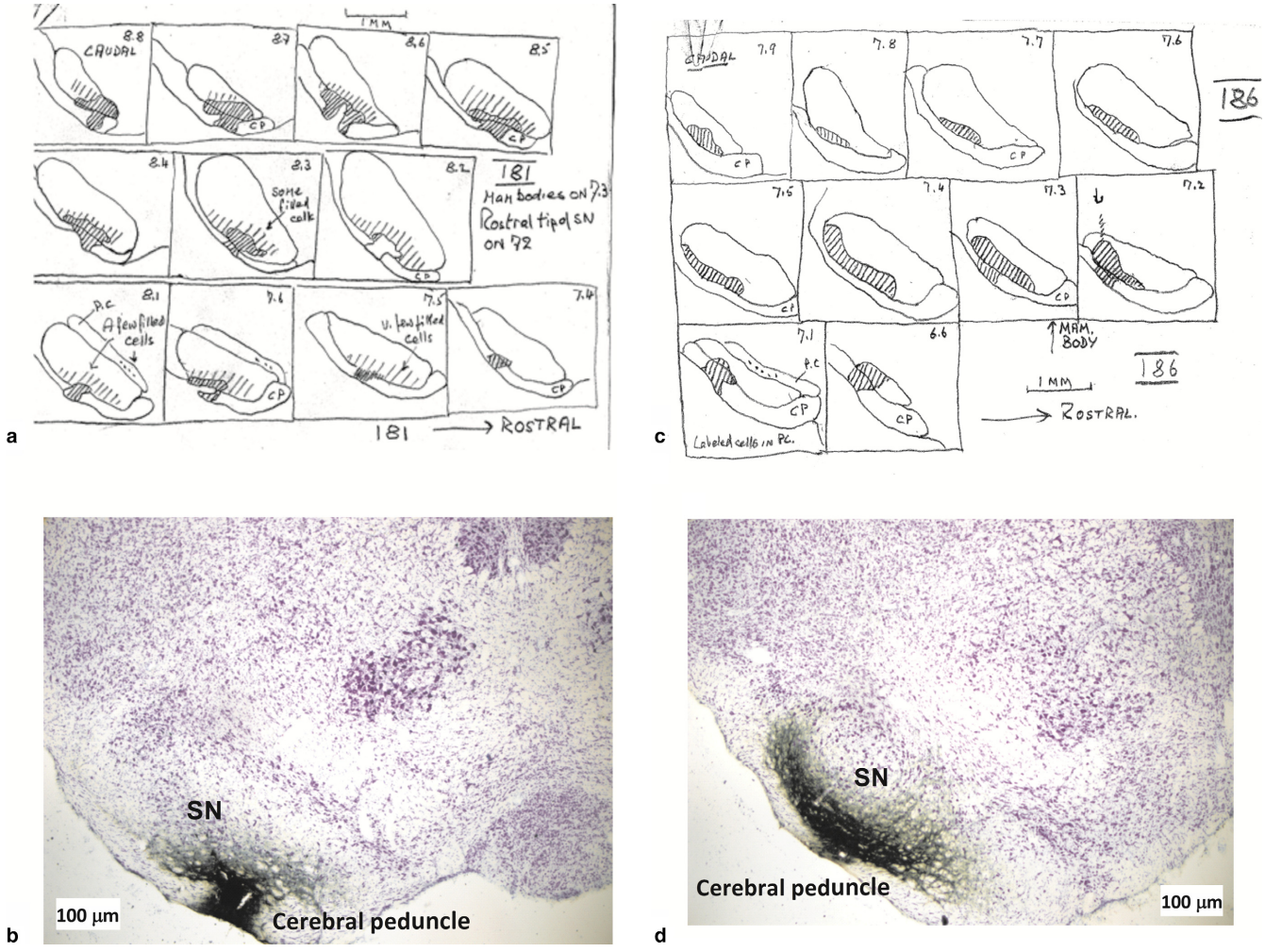

FIG. 1. The BDA injections; (a and c) Schematic illustrations and (b and d) photomicrographs of the sites of injection of BDA into the substantia nigra that included contamination of the cerebral peduncle in experiments 181 and 186. The schematics in a and c are original drawings of Ray Guillery.

three books with Murry Sherman covering those topics. He was very enthusiastic about his most recent book (Guillery, 2017). He did not hesitate to advocate anything that he believed was correct, in one of his emails he wrote "*I am not sure what reactions I will get. Some signs are that the book will puzzle, possibly even antagonize some people. But I can't worry about that.*" In his last emails, he was so anxious to finish the book and had made all plans regarding his life after the completion of the book. He wrote "*I am beginning to think about moving into a home for old academics here in Oxford, where I can be fed by other peoples' cooking and where I can have more company than I have here. Something I may need once I stop worrying about the book.*" He dedicated his life to solving:

> the puzzle of just how we relate to the world around us …. that has long concerned philosophers, psychologists and occasionally also neuroscientists. How do we know the world? Do we know the objects of the world, the apples, trees, people, houses, directly as primary objects that we identify on the basis of the lights, sounds, smells, tastes, touches, temperatures or pressures that we receive from the world, or do these, our perceptions of the sensory inputs, provide the primary and only information we have about the world? Do we first know the world, or our perceptions? (Guillery, 2017)

FIG. 2. Original schematic illustrations drawn by Ray Guillery showing labelled corticofugal axons (Experiment 181).

In the 1970s, Ray had noticed that the thalamus not only acts as a relay (for a message coming from outside and then relayed in the thalamus) but also acts as a modulator (receiving a message from one layer of the cortex and sending this information to the another layer of cortex).

Ray gave an enormous amount of support to one of our projects entitled "Topographical connections of the substantia nigra pars reticulata to higher order thalamic nuclei in the rat" (Gulcebi et al., 2012), in which biotinylated dextran amine (BDA) was used as a tracer. Rather than the projections of substantia nigra pars reticulata, he was excited to observe corticofugal axons giving branches to ventral anterior and ventral lateral nuclei of the thalamus, as higher order thalamic nuclei, in those cases in which the BDA contaminated the cerebral peduncle. Two of the BDA injections (Experiments 181 and 186) showed clear evidence of labelling in the cerebral peduncle, ventral to the substantia nigra pars reticulata (Figure 1), and one of the BDA injections (Experiment 187) was directly into the cerebral peduncle without labelling in the substantia nigra pars reticulata. To

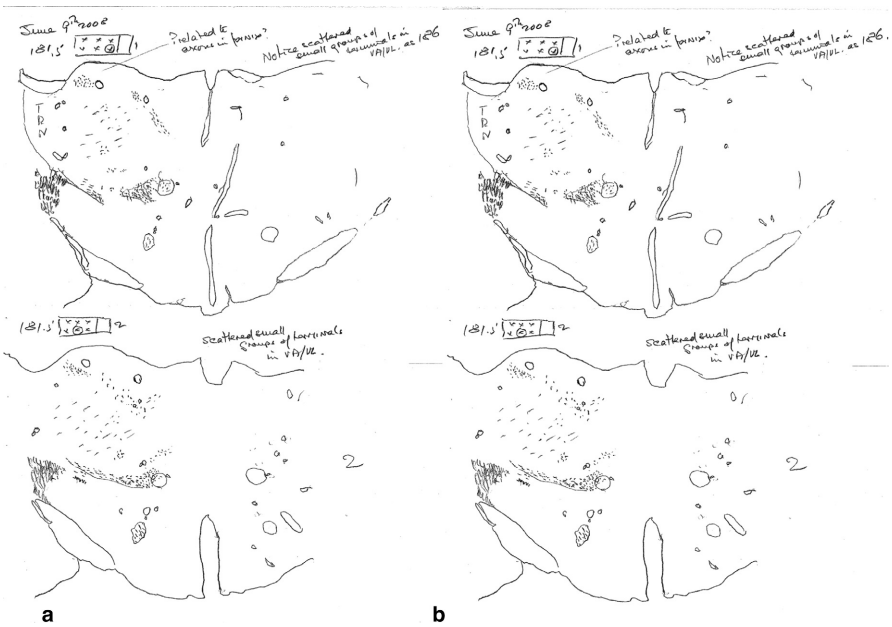

FIG. 3. Original schematic illustrations drawn by Ray Guillery showing BDA labelled terminals distributed primarily in the ventral anterior (VA) and ventral lateral (VL) nuclei of the thalamus (Experiment 181).

highlight the distribution and the borders of the BDA labelling, slides were restained with thionine by the Nissl method (Figure 1b and d). These three BDA injections showed labelled corticofugal axons caudal to substantia nigra pars reticulata (Figure 2) and also showed well-labelled cells in layer 5 of the ipsilateral cortex. There were many retrogradely labelled layer 5 cortical cells and relatively large terminals distributed primarily in the ventral anterior-ventral lateral nuclei with a clear border between these and the ventral posteromedial nucleus. No labelled terminals were observed in the ventral posteromedial nucleus (Figures 3 and 4). Ray described the detail and the importance of these findings as follows:

In what follows I provide a fuller explanation as to why I regard these results as important and possibly worth following up.

Two experiments [181 and 186] showed significant (and very beautiful) retrograde labeling of cortical layer 5 pyramidal cells with apical dendrites often extending to layer 1 in frontal and medial areas of the cortex. These labeled terminals did not encroach upon the ventral posterior nucleus, leaving a clear border between the labeled ventral anterior-ventral lateraland the unlabeled ventral posteromedial regions. When the axons were traced from these thalamic regions they ran towards the labeled axons that were heading towards the cortex. We regard them as thalamic branches of the retrogradely labeled cortical cells. Experiment 187, which included label of the cerebral peduncle, but had no clear label of substantia nigra pars reticulata, also shows the large terminals in ventral anterior-ventral lateral and intralaminar nuclei and serves to strengthen the interpretation of these as thalamic branches of the cortical cells. The precise distribution of the cortical cells and of these thalamic terminals has not been plotted, but they have been excluded as possible efferents from the substantia nigra pars reticulata.

I summarize them here for convenience:

1. We know from the studies of retrogradely filled cortical cells seen after injections of horseradish peroxidase into the thalamus that many cortical layer 5 pyramidal cells project to the thalamus (Abramson & Chalupa, 1985; Gilbert & Kelly, 1975).
2. We know that there are many large terminals characteristic of "drivers" that come from the cerebral cortex and terminate in higher order relays in the thalamus. Light microscopy has shown that such large terminals come from layer 5. Electron microscopic evidence shows that characteristic large terminals from cortex go to higher order nuclei, whereas the first order nuclei, such as lateral geniculate nucleus or ventral posterior nucleus receive such inputs from ascending pathways but not from cortex (Bourassa & Deschênes, 1995; Bourassa, Pinault & Deschênes, 1995; Darian-Smith, Tan & Edwards, 1999; Deschênes, Bourassa & Pinault, 1994; Guillery, Feig & Van Lieshout, 2001; Kakei, Na & Shinoda, 2001; Ojima, 1994; Rockland, 1998).
3. Several of the studies (Abramson & Chalupa, 1985; Gilbert & Kelly, 1975) have shown that there are layer 5 cortical cells that have a branch with large terminals in higher order thalamic relays and also have a long descending branch going to brain stem or spinal cord. Apart from a study we did (Guillery et al., 2001), in which we showed that of 48 axons having characteristic large "driver" terminals in the cat's lateral posterior nucleus all (or almost all) also had a descending branch going to brain stem, there is no evidence about the numerical extent to which the thalamic branches are always, commonly or only rarely branches of descending brainstem axons. Currently we know very little about the numerical significance of the branched axons, as compared to what is known about the importance of a layer 5 input to thalamus. There is not much

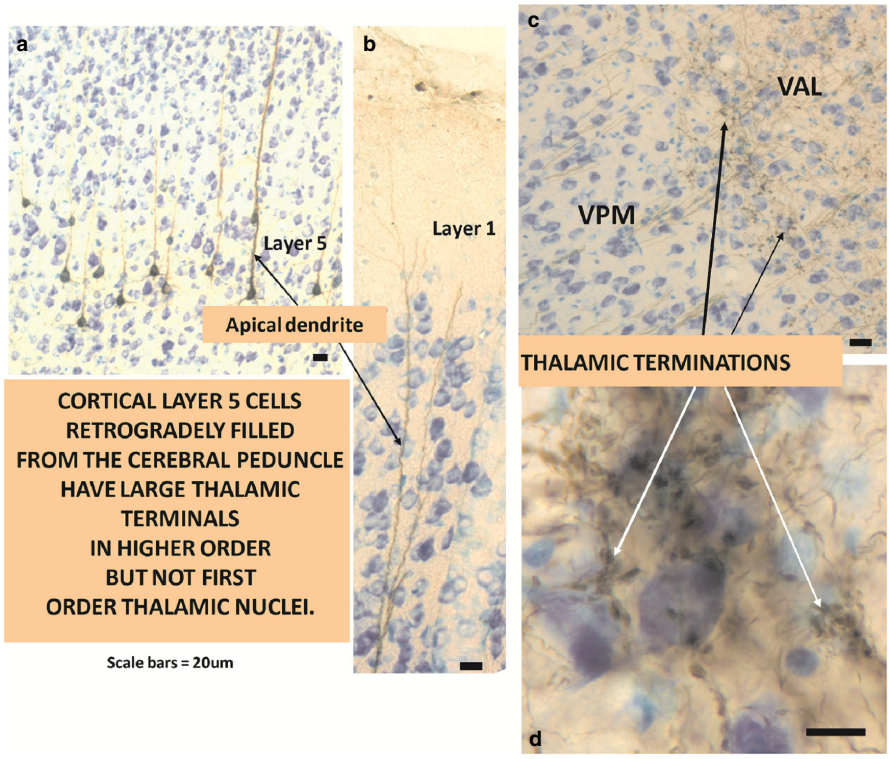

FIG. 4. Original photographs showing BDA labelled cortical cells and ventral anterior-ventral lateral (VAL) terminals were taken by Ray Guillery and Medine Gülçebi. (a) The BDA labelled cortical layer V cells, (b) magnified photograph of cortical layer 5 cells with their apical dendrites extending into cortical layer 1, (c) densely labelled ventral anterior-ventral lateral (VAL) terminals, (d) magnified photograph of large VAL terminals. VPM, ventral posteromedial nucleus.

evidence for any higher order relay to indicate how common this branching pattern is in the corticothalamic pathways that go from layer 5 to the higher order thalamic relays.
4. The strikingly dense distribution of large terminals in parts of ventral anterior-ventral lateral in these two rats suggests that the branching pattern is very common, if the terminals can all be treated as coming from branches of the corticofugal axons filled in the cerebral peduncles ventral to the substantia nigra pars reticulata.
5. Rough counts indicate that in the regions of densest terminations there are often up to 20 terminals per Nissl stained thalamic cell. This is lower than a figure that can be derived for number of retinal driver inputs in the lateral geniculate nucleus of the cat (20–40 is a rough estimate that Murray Sherman sent me recently). However, we have probably not filled all of the relevant (thalamus projecting) layer 5 cells, and we are probably looking at a thalamic nucleus that is mixture of first order inputs from cerebellum and higher order inputs from cortex. A comparison with lemniscal inputs to the ventral posteromedial could be instructive.

For me this material is important because a cell that simply passes a message from the cortex to the thalamus is telling the thalamic relay about ongoing cortical computations that may or may not lead to an action. A cell with a branching axon may carry comparable information about the cortical computation but in addition it is also telling the thalamic relay that a particular motor instruction has already been initiated. I have been working hard to try to persuade colleagues about the importance of such branching patterns, but with almost no success. These brains (if my interpretation of them is correct) tell us that such branched axons play a major role in thalamocortical organization.

There was not much evidence that showed ventral anterior and ventral lateral as a first order or higher order thalamic nuclei. The observations provided evidence that ventral anterior and ventral lateral nuclei are mixed higher order thalamic nuclei. Thus, they receive the well-known driving afferents from the cerebellum and the present observations showing driving afferents from the cortex. On the basis of current evidence, it thus seems likely that there will be several cell groups that represent a mixture of first and higher order circuits. Where such mixed inputs are identified, an important next question will be whether first and higher order relays innervate common thalamic relay cells or whether within one nucleus they are kept as separate and parallel pathways going through the nucleus independently or can there be integrative interactions between first and higher order relays? The driver afferents sending axonal branches to motor or premotor centres are important aspects of the perceptual processes (Guillery, 2003). The driver afferents to the first order thalamic nuclei sending branches to motor or premotor centres of the brainstem are well documented; however, the driver afferents to the higher order thalamic nuclei sending branches to motor or premotor centres are not well characterized. In his observations, Ray also found evidence for branching of the driver afferents to the higher order thalamic nuclei.

"Finding the right questions" is the most important challenge that reflects the impact of Ray on our lives. Ray provided us with significant vision, not only for the characterization of scientific activity and complicated interactions within the brain, but also for the interrogation of our existence in the world, aims and future dreams. We trained every day under Ray mentorship during his stay in Marmara University. He contributed greatly to the development of neuroscience both in Marmara University and the neuroscience society from various parts of Turkey. After he left Marmara University and Istanbul, we kept numerous documents, writings and illustrations of Ray, including documents in the present manuscript. Here, we have tried to convey his excitement about his personal observations of branched axons and the possible importance of the role of these branched axons in the thalamocortical organization within the framework of his and our reflections of the period we lived with Ray. We are very pleased and privileged to have experienced and interacted with Ray who was a great role model as a neuroscientist and academician, with a sharp naïve mind and wonderful sense of humour. We deeply miss Ray, the beautiful mind. In summary, this manuscript is a typical but unique story of Ray that should be told. Dearest Ray, we thoroughly thank you for spending those precious years with us.

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