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# Cortical contribution to sensory volleys recorded at thalamic nuclei of lemniscal and paralemniscal pathways

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Short communication

**Abstract.** In order to elucidate the role of cortical input on sensory information processing in different thalamic somatosensory nuclei we recorded potentials evoked (EPs) by whisker deflections of short duration from ventral posteromedial (VPm) and medial posterior (POm) nuclei while manipulating cortico-thalamic activity by means of local cooling, lidocaine application or electrical stimulation. It appeared that only the earliest sub-component of the first negative wave of the EPs resulted from peripheral input, while the rest of the potential's negativity depended on cortical feedback. The latencies and amplitudes of EPs recorded at both nuclei were not significantly different, which might be attributed to urethane anesthesia.

**Key words:** vibrissa/barrel system, cortico-thalamic projection, evoked potentials, cortical inactivation, rat

Tactile information from the whisker receptors of the rat's snout among other thalamic targets reaches two dorsal nuclei: ventral posteromedial (VPm) and medial posterior (POm), which for a long time have been regarded as belonging to so called "lemniscal" and "paralemniscal" pathways, respectively (Diamond et al. 1992). The VPm contains neuronal groupings called barreloids which are activated primarily by inputs from individual whiskers and project mostly, although not exclusively, to individual barrel columns of the primary somatosensory cortex (the barrel cortex). The VPm neurons receive in turn cortico-thalamic fibers from cortical layer 6. Less precise somatotopic organization can be found in POm and the projection from this nucleus to the barrel cortex (and to other cortical areas) is also more diffuse (Deschênes et al. 1998). Unlike VPm, POm receives fibers originating in two cortical layers: 5 and 6. These two groups of afferents differ morphologically and are believed to play different functional roles. It has been proposed that the projection from layer 6 may subserve gain control at the thalamic level (Lindstrőm and Wróbel 1990). On the other hand, layer 5 cortico-thalamic fibers have been hypothesized to provide thalamic neurons with the strongest, "driving" input (Sherman and Guillery 2002). In order to study the cortical share in evoked potentials recorded in VPm and POm after whisker stimulation, we manipulated the activity level of the barrel cortex by cooling, topical lidocaine application and/or direct electrical stimulation.

The experiments were performed on 8 adult male Wistar rats weighing 330-390 g. The animals were anesthetized with urethane (1.5 g/kg i.p., with 10% of the original dose added when necessary) and placed in a stereotaxic apparatus. Fluid requirements were fulfilled by s.c. injections of 0.9% NaCl (1 ml per hour). The body temperature was monitored during the whole experiment and maintained in physiological range with a heating blanket. Lidocaine was applied into the rats' ears (Lignocainum hydrochloricum 2% gel, Jelfa, Poland) and injected into the skin over the skull (Xylocaine 2%, Astra, Sweden) prior to surgery. Two openings in the skull were made: one exposing the dura mater overlying right VPm and POm, and the other over the right barrel field. The left whiskers were glued together to a thin needle (10-15 mm from the snout) mounted on a piezoelectric slab. Square wave pulses of 2 ms duration delivered from a PC produced 0.1 mm vertical movements of the whisker bunch at pseudo-random intervals of mean frequency 0.2 Hz. All procedures were approved by the Ethics Committee for Animal Experimentation.

Both thalamic and cortical electrodes (impedance of 100 kOhm at 10 kHz) were made in our laboratory from insulated 25 μm tungsten wires with sharpened tips. They were used for monopolar local field potential (LFP) recording against a reference electrode prepared from Ag-AgCl wire and placed between the skull and overlying soft tissues. Thalamic electrodes aiming at VPm and POm were lowered simultaneously using stereotaxic coordinates (AP = -3.3 mm from bregma, L = 2.2-2.8 mm from midline; Paxinos and Watson 1997) and their position controlled by monitoring on-line cellular activity evoked by visual and tactile stimuli. An additional multielectrode, lowered perpendicularly to the cortical surface at the center of the barrel field (AP = -2.5 mm from bregma, L = 5.8 mm from midline), allowed recordings from 3 to 4 different cortical depths. Electrodes crossed the dura through small holes while the rest of intact dura in the skull openings was covered with humid Spongostan (Ferrosan, Denmark). The LFP signal was amplified (1,000 x), filtered (0.1 Hz-5 kHz) and stored on magnetic tape of RACAL V-store recorder (Racal Recorders Ltd, England). Epochs (of about 1.4 s) containing potentials evoked (EPs) by whisker stimulation were digitized on-line (10 kHz) with 1401plus interface and Spike2 software (CED, Cambridge, England). All stored data were examined for integrity and epochs with artifacts were excluded from further analysis.

We changed the barrel cortex temperature by washing overlying dura with gradually cooled and rewarmed saline. The EPs were recorded when the saline temperature was stabilized at 37°C, 10°C and 4°C (as measured in close vicinity of the skull opening). Lidocaine (Xylocaine 2%, Astra, Sweden) was applied by dripping at Spongostan covering the dura (at room temperature) and then washed out. The thalamic evoked potentials were also elicited from the cortical site by electric stimulation through deep cortical electrodes (single pulse, 0.05 ms, 0.1-0.25 mA).

After completion of an experiment small electrolytic lesions were made through the recording electrodes, the animals were killed by overdose of pentobarbital sodium (Nembutal, Abbot Laboratories, USA, 1 ml i.p.) and perfused transcardially with saline followed by 4% formalin. Their brains were removed, kept for at least two days in formalin-sucrose solution and then cut to 50 μm slices and stained with typical Nissl procedure. The

exact position of electrode tips was verified histologically and the lesions drawn with use of camera lucida.

Local cooling of the cortical surface resulted in various changes of EPs recorded in the barrel cortex (Fig. 1). It must be stressed that temperature of the cortex was probably higher than that of the saline above the dura and the cooling effect was gradually less pronounced with cortical depth (Kublik et al. 2001). Lowering of superficial temperature to 10°C led to significant, albeit partly transient, increase of the amplitudes of cortical EPs, especially prominent for recordings from infragranular layers. These observations reflect an underlying increase of summed postsynaptic potentials (PSPs) within contributing cortical neurons. The enhancement of summed PSPs might result from disinhibition and/or be directly caused by cooling. Moderate cooling of superficial cortical layers could strongly affect the pool of inhibitory interneurons either because of their higher density in the superficial layers (Keller 1995) or their putative special vulnerability toward lowered temperature. Disinhibition may also speed up the synchronization processes and thus enhance the EPs amplitudes. An alternative scenario may include cooling-induced changes at the synaptic and cellular level which may slow down PSPs' time-courses, prolong the period of temporal summation (Volgushev et al. 2000a) and thus can also increase the amplitude of evoked responses. In this respect it is worth noting that peak latencies of cortical EP waves increased in lower temperatures (Fig. 1). Prominent early

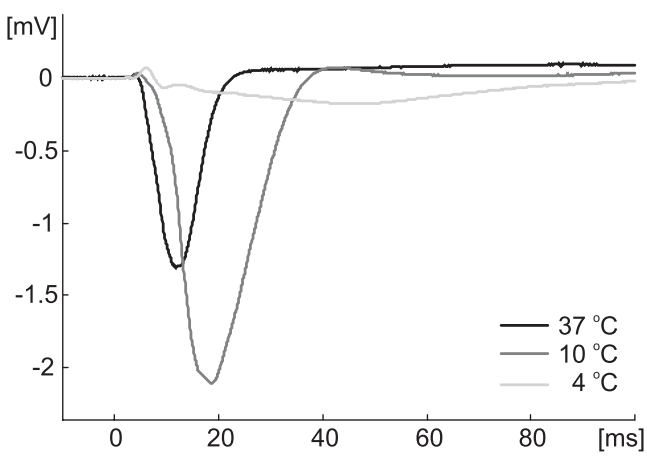


Fig. 1. Averages (over 60 trials) of potentials evoked by vibrissal stimulation in the infragranular layers of the barrel cortex at physiological temperature and during local, superficial cooling to 10°C and 4°C.

negative wave of EPs recorded in the infragranular layers was always accompanied by simultaneous multiunit activity (not shown). We can, therefore, assume that larger amplitude of this wave after moderate (10°C) cooling resulted also in higher cortico-thalamic activity. Indeed, increased cortical activity after moderate cooling was described previously in rat (Gartside and Lippold 1967) and cat (Adey 1974, Moseley et al. 1972), and cellular mechanisms of increased neuronal activity during moderate cooling have been proposed (Volgushev et al. 2000b).

Lowering the temperature of the cortical surface to 4°C led to complete blockade of evoked activity in supragranular layers and significantly decreased, residual activity in infragranular layers (Fig. 1). This, in turn, should lower the activation in cortico-thalamic pathways. Thus, the diverse effects of moderate and strong cortical cooling gave us the opportunity to evaluate dependence of thalamic EPs to both increased and decreased cortical inputs.

Thalamic EPs were reasonably discernible only after averaging of about 50 responses and were composed of a characteristic sequence of positive and negative waves (Fig. 2A-D,F). Similar to cortical electrodes, some thalamic electrodes also recorded multiunit neuronal activity together with lower frequency LFPs. This allowed us to attribute the negative waves (N1 and N2 at Fig. 2F) of the EPs to increased neuronal activity in thalamic sites. The dropping arm of the first negative wave (N1) was typically composed of two phases (N1.1 and N1.2) with clearly discernable switching point as indicated by arrows in Figs. 2A and 3.

Neither the amplitudes of consecutive waves nor their latencies were found to be significantly different between VPm and POm evoked potentials (see Fig. 2E). A difference that could be assessed only qualitatively was the presence of more prominent notches (indicated by dots in Fig. 3) observed regularly at the initial phase of the first negative wave (N1.1) in VPm as compared to POm. Given that peaks in post-stimulus-time-histograms of multiunit activity occurred with similar latencies as these notches (not shown), it seems reasonable to propose that they corresponded to population spikes. Indeed, the two notches observed in each record most probably represented ON and OFF responses for vibrissal deflection since the gap between them elongated accordingly when longer ramp-and-hold stimuli were applied. Note that the putative population spikes overlapped on the initial phase of N1.1, which should

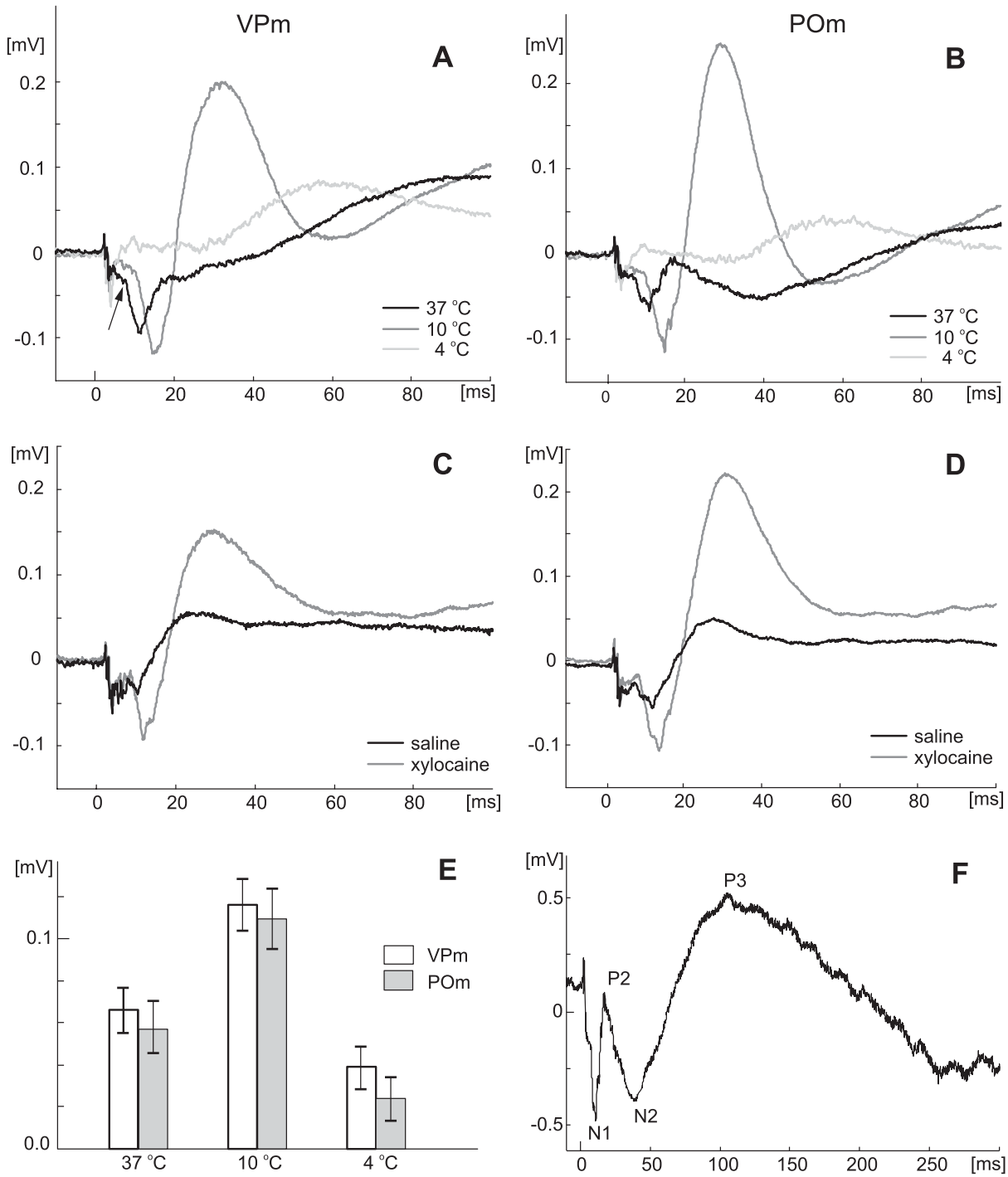


Fig.2. (A-D), (F), Somatosensory evoked potentials (EPs) recorded from the thalamic nuclei of the same rat during single experiment. The EPs (50 averaged traces) were recorded in VPm (A, C) and POm (B, D). (A), (B) Cooling experiment: recordings were carried out at three stabilized temperatures (37°C, 10°C and 4°C as measured at the surface of the barrel cortex). (C), (D) Recordings before and after lidocaine application on the barrel cortex (at room temperature). (E) Averaged amplitudes (of 4 animals) of N1 measured from VPm and POm recordings at physiological temperature and during superficial cooling of the barrel cortex to 10°C and 4°C; ± standard deviation. (F) Typical averaged EP recorded in POm at physiological temperature (note the longer time-scale). Positive waves are designated with letter P and consecutive numbers, negative waves with letter N. The effects of cooling and lidocaine application were fully reversible. An example of a border between sub-components of N1 (i.e., N1.1 and N1.2) is indicated by an arrow in (A).

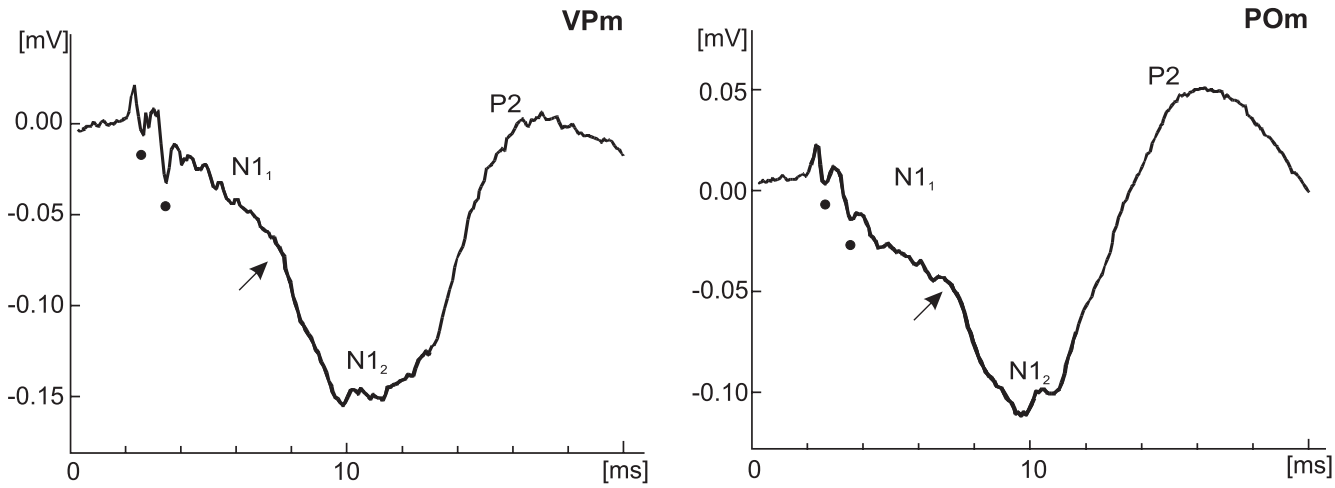


Fig. 3. Examples of averaged EPs recorded in VPm and POm at physiological temperature, with marked border between sub-components of N1 wave. N1.1 corresponds to peripheral and N1.2 to cortical origin component. Notches, representing population spikes, are indicated by dots.

represent the rising phase of population excitatory PSPs at the thalamic nuclei. Since equivalent notches on N1.1 recorded in POm were wider and smaller in amplitude it could be speculated that initial responses in POm were less synchronized. Since average latencies of the earliest VPm and POm responses were found to be similar it is reasonable to postulate that both were evoked by direct peripheral inputs. This reasoning questions the former hypothesis that POm could be triggered to action only by cortical input (Diamond et al. 1992, Sherman and Guillery 2002).

Moderate cooling of the barrel cortex (10°C), which increased cortico-thalamic activity during negative waves of cortical EP, resulted also in significant amplitude increase of corresponding components of thalamic EP, including the later phase of the first negative wave (N1.2; Fig. 2A,B and E) whereas the initial phase remained unchanged. Similarly, strong cortical cooling (4°C) that resulted in decreased activity of the cortico-thalamic neurons and attenuated all waves of the EPs recorded in thalamic nuclei, did not change the initial phase of their first negative wave (N1.1) containing the population spike notches and attributed, by previous reasoning, to the peripheral excitation. It seems, therefore, justified to propose that the two discernable phases at the dropping arm of first negative wave of thalamic EP manifest its genuine sub-components (Fig. 3). The first sub-component (N1.1 in Fig. 3) would thus represent peripheral input, while the second one (N1.2 in Fig. 3), which mirrors the changes of cortico-thalamic activity, could have been attributed to cortical input. Our hypothesis was further supported by the finding that stimulation of the barrel cortex by means of electric current produced thalamic EP with first negative component of the latency which was equal to difference between latency of N1.2 component of thalamic potential and latency of N1 wave of cortical EP, both evoked by vibrissal stimulation (not shown). Our conclusion clearly differs from that presented recently by Temereanca and Simons (2003) who regarded the whole thalamic N1 wave (which they called the early component of the local field potential) as representing solely peripheral input. Other positive and negative waves of thalamic EPs, following N1 (Fig. 2F) changed as a function of cortical temperature (Fig. 2A,B) and thus represented a mixture of higher order reentrant activities, originating presumably in the barrel cortex and thalamic reticular nucleus.

In contrast to all other waves of cortical evoked potentials which were delayed during the cooling experiment, the latencies of earliest sub-components (N1.1) of thalamic EPs varied insignificantly (by less than 0.5 ms) in the same circumstances. This observation indirectly supports the notion that thalamic temperature remained stable during the cooling experiment. Nevertheless, some previous results do not completely exclude the possibility of spread of the temperature reduction from the cortical surface into the thalamus (Yuan et al. 1985). Without the ability to measure thalamic temperature we decided to perform control experiments inducing corti-

cal inhibition by use of lidocaine. Application of 2% lidocaine on the cortical surface appeared to increase amplitudes of cortical EP waves, similarly to moderate cortical cooling, which favors the hypothesis linking such an increase with inhibition of superficially located inhibitory interneurons. The lidocaine application also resulted in increased amplitudes of thalamic EP waves with the exception of the shortest, peripheral N1.1 (Fig. 2C,D).

In both experiments described above, when changes of cortical activity were produced by cooling or lidocaine application, we did not find any significant differences between amplitudes of EP waves evoked simultaneously in VPm and POm (Fig. 2E). This finding might be explained by assuming that urethane anesthesia affected the activity of cortico-thalamic neurons of layer 5 in a way that diminished their influence on the thalamus, and that similar cortico-thalamic activity is transmitted to both nuclei *via* afferents originating in layer 6. Further experiments are needed to clarify this hypothesis.

## CONCLUSION

The first negative wave of potentials evoked in lemniscal and paralemniscal somatosensory thalamic nuclei by whisker stimulation is composed of two components representing peripheral and cortical inputs.

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Adey W.R. (1974) Biophysical and metabolic bases of cooling effect on cortical membrane potentials in the cat. Exp Neurol 42: 113-140.

Deschęnes M., Veinante P., Zhang Z.W. (1998) The organization of corticothalamic projections: reciprocity versus parity. Brain Res Rev 28: 286-308.

Diamond M.E., Armstrong-James M., Ebner F.F. (1992) Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus. J Comp Neurol 318: 462-476.

Gartside I.B., Lippold O.C.J. (1967) The production of persistent changes in the level of neuronal activity by brief local cooling of the cerebral cortex of the rat. J Physiol 189: 475-487.

Keller A. (1995) Synaptic organization of the barrel cortex. In: Cerebral Cortex Vol. 11: The Barrel Cortex of Rodents (Eds. E.G. Jones and I.T. Diamond). Plenum Press, New York, pp. 221-262.

Kublik E., Musiał P., Wróbel A. (2001) Identification of principal components in cortical evoked potentials by brief surface cooling. Clin Neurophysiol 112: 1720-1725.

Lindstrőm S., Wróbel A. (1990) Frequency dependent corticofugal excitation of principal cells in the cat's dorsal lateral geniculate nucleus. Exp Brain Res 79: 313-318.

Moseley J.I., Ojemann G.A., Ward A.A. Jr (1972) Unit activity during focal cortical hypothermia in the normal cortex. Exp Neurol 37: 152-163.

Paxinos G., Watson C. (1997) The rat brain in stereotaxic coordinates. Compact third edition. Academic Press, Inc., San Diego

Sherman S.M., Guillery R.W. (2002) Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. Neuron 33: 163-175.

Temereanca S., Simons D.J. (2003) Local field potentials and the encoding of whisker deflections by population firing synchrony in thalamic barreloids. J Neurophysiol 89: 2137-2145.

Volgushev M., Vidyasagar T.R., Chistiakova M., Eysel U.T. (2000a) Synaptic transmission in the neocortex during reversible cooling. Neuroscience 98: 9-22.

Volgushev M., Vidyasagar T.R., Chistiakova M., Yousef T., Eysel U.T. (2000b) Membrane properties and spike generation in rat visual cortical cells during reversible cooling. J Physiol 522.1: 59-76.

Yuan B., Morrow T.J., Casey K.L. (1985) Responsiveness of ventrobasal thalamic neurons after suppresion of S1 cortex in the anaesthetized rat. J Neurosci 5: 2971-2978.

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