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ELECTRICAL PROPERTIES OF MORPHOLOGICALLY CHARACTERIZED NEURONS IN THE INTERGENICULATE LEAFLET OF THE RAT THALAMUS

H. J. SZKUDLAREK[a] AND M. RAASTAD[b]*

[a]*Department of Neurophysiology and Chronobiology, Chair of Animal Physiology, Institute of Zoology, Jagiellonian University, R. Ingardena Str. 6, Krakow 30-060, Poland*

[b]*Institute of Basic Medical Sciences, University of Oslo, Box 1103, Blindern, 0317 Oslo, Norway*

Abstract—The intergeniculate leaflet (IGL) is a flat thalamic nucleus that responds to retinal illumination, but also to non-photic input from many brain areas. Its only known function is to modulate the circadian rhythm generated by the suprachiasmatic nucleus. Previously, the firing behavior of cells in IGL has been investigated with extra-cellular recordings, but intracellular recordings from morphologically identified mammalian IGL neurons are lacking.

We recorded from and labeled IGL cells in rat brain slices to characterize their basic membrane properties and cell morphology. A high fraction of neurons (82.5%) were spontaneously active. The silent cells were identified as neurons by electrophysiological techniques. The spontaneous activity was due to intrinsic membrane properties, and not driven by rhythmic synaptic input. Most spontaneously active cells had a very regular firing pattern with a coefficient of variation of the spike intervals <0.12 in more than 50% of the cells. Rebound depolarization after a hyperpolarizing pulse, usually with one fast action potential on top, was observed in 80% of the cells.

The silent neurons had a range of resting membrane potentials and spike thresholds overlapping with the active ones. This suggests that spontaneous activity was controlled by several, yet undetermined factors in addition to membrane potential.

Within the IGL we found a broad range of morphologies without apparent categories and no significant correlation with activity. However, the spontaneous, usually regular, spiking and the rebound depolarization of IGL cells is typical a feature that distinguish them from neurons in ventral and from interneurons in the dorsal lateral geniculate nuclei.

Key words: intergeniculate leaflet, thalamus, patch clamp, morphology, circadian rhythms.

The intergeniculate leaflet (IGL) is a flat structure localized between the dorsal and ventral parts of the lateral geniculate body (dLGN and vLGN, respectively). It can be distinguished from these adjacent nuclei because many of its cells contain neuropeptide Y (NPY) or other peptides, and glutamic acid decarboxylase (GAD) (Moore and Card, 1994; Morin and Blanchard, 2001). Until 1976 (Hickey and Spear, 1976) the IGL was described as a part of vLGN, and still little is known about the electrical activity of its cells.

Despite rich innervations from the retina (Moore and Card, 1994; Hattar at al. 2006) and other brain nuclei that receive retinal input (Morin and Blanchard, 1998, 2001; Moore et al., 2000), the IGL has no known function in image processing. The single established IGL function (but not necessarily the only function, see i.e. Harrington, 1997; Morin and Blanchard, 1998, 2005) is the contribution to modulation of circadian rhythms (Harrington, 1997; Morin and Allen, 2006), mainly through its reciprocal connections with neurons within or close to the circadian rhythm generator located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Card and Moore, 1989; Moore et al., 2000).

Several lines of evidence indicate that IGL is important for circadian rhythm modulation. First of all, lesions of the IGL or its connections interfere with how light and other stimuli influence circadian rhythms (Harrington and Rusak, 1986; Pickard et al., 1987; Johnson et al., 1988, 1989; Janik and Mrosovsky, 1994; Maywood et al., 1997; Edelstein and Amir, 1999).

In vivo, extracellular recordings show that many IGL neurons code illumination level (Harrington and Rusak, 1991). This has been suggested to occur, at least partly, via melanopsin-containing retinal ganglion cells, considered circadian photoreceptors (Hattar et al., 2002, 2006; Morin et al., 2003). Retinal illumination also induces c-Fos activity in IGL cells (Janik et al., 1995; Edelstein and Amir, 1996; Peters et al., 1996; Muscat and Morin, 2006), sometimes in conditions that do not induce c-Fos activity in the SCN (Edelstein and Amir, 1996; Muscat and Morin, 2006).

Many IGL neurons recorded *in vivo* display a tonic, regular firing (Harrington and Rusak, 1989), preserved in slice preparations, showing that some of the IGL activity is intrinsic to the nucleus (Lewandowski and Blasiak, 2004). Spontaneously active cells in the slice have been classified as regular, irregular or bursting (Blasiak and Lewandowski, 2004), but the slow oscillation in spike frequency, described both in dark (Harrington and Rusak, 1989) and light-stimulated conditions *in vivo* (Lewandowski et al., 2000) is missing in the slice preparation.

From the above references it is clear that spontaneous spike activity, both *in vivo* and in the isolated preparation,

*Corresponding author. Tel: +47-22-85-12-96.
E-mail address: morten.raastad@basalmed.uio.no (M. Raastad).
Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; CC, current clamp; CV, coefficient of variation; cvISI, coefficient of variation interspike interval; dLGN, dorsal part of the lateral geniculate body; GAD, glutamic acid decarboxylase; IGL, intergeniculate leaflet; ISI, interspike interval; NPY, neuropeptide Y; $perc_{10\text{-}90}$, 10th and 90th percentiles; Rin, input resistance; SCN, suprachiasmatic nucleus; vLGN, ventral part of the lateral geniculate body.

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is more typical in IGL than in the adjacent nuclei. This activity may therefore be important for IGL function and it would be interesting to know if the spontaneous activity is a feature of a particular subpopulation of neurons. Although some silent neurons can be stimulated to fire with extracellular methods, intracellular recordings are usually necessary to be sure a cell can fire spikes. Also, because systematic intracellular recordings are lacking, it is not known whether the firing patterns are driven by synaptic activity intrinsic to the nucleus, or, alternatively, by the membrane properties of the individual cells. Intracellular recordings are also helpful in characterizing firing patterns because they exclude the contamination of spikes arising from more than one unit.

For these reasons, and to give basic electrical and morphological characteristics of IGL neurons, we here provide intracellular recordings from morphologically identified IGL neurons.

EXPERIMENTAL PROCEDURES

Preparation

All experimental procedures were approved by the National Animal Research Authority in Norway, which conforms with European regulations. Every effort was made to minimize the number of animals used and their sufferings. Wistar rats (age range 9–21 and 9–90 days old for morphological and electrophysiological data, respectively) were anesthetized with Suprane (Baxter, Oslo, Norway) and decapitated. Animals were killed between 10 AM and 1 PM and experiments performed between 1 and 9 PM. The animals stayed with their mothers at least 1 week in our animal facility and light was on between 7 AM and 6 PM. Blocks of brain tissue were quickly removed, submerged in ice-cold, oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) containing the following (in mM): NaCl 125, KCl 1.25, $CaCl_2$ 2, $MgCl_2$ 1, $NaHCO_3$ 25, KH_2PO_4 1.25, D-glucose 25, at pH 7.4 and placed on a cold plate of a vibroslicer (Campden Instruments, Loughborough, UK) where coronal slices (300 μm thick) containing the IGL were cut. Slices were trimmed, bisected at the midline and placed in a holding chamber for at least 45 min before they were transferred to the recording chamber where they were kept submerged. The oxygenated ACSF solution in the holding and recording chambers was kept at room temperature (27±0.5 °C). The IGL was identified using bright field microscope with 4× objective.

Electrophysiology

Patch electrodes were made from borosilicate capillaries (Harvard Apparatus, Holliston, MA, USA) and had resistance of 7–10 MΩ when filled with (in mM): potassium gluconate 115, Hepes 10, adjusted to pH 7.3. Liquid junction potential was approximately −13 mV and was added to the measurements of membrane potentials. The blind method (Blanton et al., 1989) was used to obtain GΩ seals. The electrode capacitance was compensated before the membrane was ruptured by negative suction and the zap function. Whole-cell recordings from the somata of the IGL neurons were made in current clamp (CC) and voltage clamp (VC) modes with an Axopatch 1D amplifier (Axon Instruments, USA) without serial resistance compensation or bridge balance. Stimulus delivery and data acquisition were performed using custom-made software. For CC experiments we used 100 ms long steps, polarizing the membrane in six to eight steps between −60 and +40 mV from holding potential (usually close to the resting potential). Resting membrane potential was estimated off-line for silent cells and for cells with silent periods. Cells with resting potentials more positive than −30 mV (−43 mV when adjusted for the junction potential) were discarded.

The apparent cell capacitance and membrane time constant were found by fitting exponential functions, usually the sum of two, to the charging curves of the voltage and current response to square steps of current and voltage, respectively. Usually four to six different step amplitudes were fitted, and the median value for the estimated parameter selected. To find the best fits, we minimized the squared error between the data and the functions.

The total cell capacitance was estimated as the time integral under the capacitance transient divided by the voltage step. The integral was found by fitting the sum of two exponential functions.

The membrane time constant was estimated in CC as the slower of two fitted exponential functions to the charging curve in response to a square current step. In some cases a step function was added to account for the electrode access resistance. The optimal fits were found by minimizing the squared deviations between the functions and the data.

Rebound depolarization was detected as a depolarization >5 mV above baseline following a longer than 100 ms current-induced hyperpolarization. In the case of spontaneously active neurons, the cells were hyperpolarized by current injection to a level just below firing threshold, before the hyperpolarizing pulses were applied.

For firing pattern analysis spike activity was measured during the first 5–10 min of whole cell recording.

Histology and morphological reconstructions

Recorded cells (usually one per slice) were filled with biocytin (0.3%) added to the intracellular pipette solution. After recording, the slices were fixed in 0.1 M phosphate buffer containing 4% paraformaldehyde for up to 2 weeks. The slices were subsequently processed for biocytin labeling (Horikawa and Armstrong, 1988) using avidin–biotin peroxidase complex (ABC-Elite Kit; Vector Laboratories, USA) and 3,3-diaminobenzidine reaction. Slices were mounted in the aqueous medium Mowiol (Hoechst AG, Frankfurt AM, Germany). Three dimensional neuron models were constructed from stained cells using the Neurolucida system (MicroBrightField Inc., USA) and a bright-field microscope (Zeiss) with 40× water immersion objective.

Statistics

Most of the measurements are given as median and 10th and 90th percentiles ($perc_{10\text{-}90}$). Differences between the means were tested with a Student's *t*-test. Correlations were examined using Pearson's correlation coefficient (*r*), with a *P*-value assuming Gaussian distributions of the parameters.

RESULTS

Morphology

Of 106 intracellularly recorded cells, 61 were successfully filled with biocytin. Cell bodies were particularly well labeled, allowing us to determine their position relative to the IGL. Fifty-two cells (85%) were characterized as having their somata within the IGL, and 40 of these (Fig. 1) were reconstructed in three dimensions (see Experimental Procedures). Axons were not reconstructed because their small diameter prevented reliable tracing.

Because this is the first study with reconstructed IGL neurons in three dimensions we give some morphological measurements of the IGL neurons. The average membrane