ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2015, 75: 27–35

Electrophysiology of GABAergic transmission of single intergeniculate leaflet neurons in rat

Katarzyna Palus, Łukasz Chrobok, and Marian H. Lewandowski*

Jagiellonian University, Institute of Zoology, Department of Neurophysiology and Chronobiology, Krakow, Poland,
*Email: marian.lewandowski@uj.edu.pl

The intergeniculate leaflet (IGL) of the thalamus constitutes a small but important part of the neural network controlling circadian activity in rodents. It appears that IGL integrates photic cues from retina with non-photic information originating from different nonspecific brain systems. Subsequently, this integrated signal is passed to the master biological clock – the suprachiasmatic nuclei (SCN). The common neurotransmitter of biological clock neural structures, the γ-amino-butyric acid (GABA) is expressed in many, if not all, IGL and SCN neurons. Whole-cell patch clamp *in vitro* electrophysiological experiments were performed in order to evaluate GABA's influence on single IGL neurons in rat. Most neurons were hyperpolarized by GABA application and this effect was caused by activation of $GABA_A$ as well as $GABA_B$ receptors. The presence of $GABA_B$ receptors in rat's IGL has been suggested for the first time.

Key words: intergeniculate leaflet, GABA, biological clock, patch clamp

INTRODUCTION

The intergeniculate leaflet of the thalamus (IGL) is a narrow brain structure interlaid between the dorsal and ventral parts of the lateral geniculate nucleus of rodents (Moore and Card 1994). These small but important bilateral structures are known to modulate circadian rhythms, staying in reciprocal connection with the master biological clock generators – the suprachiasmatic nuclei of the hypothalamus (SCN) (Moore and Card 1994, Harrington 1997). Despite the presence of a few peptides located in IGL neurons, which are a key factor in differentiating the nucleus subpopulations, all IGL cells express the γ-amino-butyric acid (GABA) (Moore and Speh 1993). The first neuronal group, distinguished by the expression of enkephalin (ENK), connects two contralateral IGLs (Takatsuji and Tohyama 1989, Harrington 1997), has a direct retinal innervation (Juhl et al. 2007) and exhibit an infra-slow (<0.1 Hz) oscillatory pattern of neuronal activity *in vivo* (Blasiak and Lewandowski 2013). The other IGL subpopulation expresses an important brain modulator – neuropeptide Y (NPY) (Harrington 1997). NPY-positive fibres originating from IGL are abundant in SCN (Card and Moore 1989, Saderi et al. 2013), and the NPY receptors coexist in pre- and postsynaptic sites on SCN neurons (Chen and van den Pol 1996). It is known that NPY can produce circadian phase shifts (Jacob et al. 1999, Lewandowski and Usarek 2002, Lall and Biello 2003). Putatively, the main function of IGL is collection and integration of the photic information from retina with non-photic cues mediated by non-specific brain systems (Harrington 1997). It has been proposed that ENK neurons modulate the NPY cells activity and the comprehensive information is relayed to the SCN (Blasiak et al. 2006). It is worth mentioning here, that all the described interplays between either IGL cells on their own, or IGL cells projecting to the SCN, and finally those within the SCN, are GABAergic in their neurochemical nature (Moore and Speh 1993).

GABA is a very common neurotransmitter in the central nervous system and the most important inhibitory substance in the adult brain (Roberts 2007). There are three types of GABA receptors: $GABA_A$ – the ligand gated chloride channel; $GABA_B$ – the G protein

Correspondence should be addressed to M.H. Lewandowski
Email: marian.lewandowski@uj.edu.pl

Received 18 August 2014, accepted 12 November 2014

coupled matabotropic receptor and $GABA_C$ (sometimes called $GABA_A$-ρ) – the ionotropic receptor abundantly expressed in retina (Bormann and Feigenspan 1995). GABA has been described as the cardinal transmitter of the mammalian biological clock, not only being present in the key structures of the time-keeping system such as the IGL or SCN (Moore and Speh 1993, Wagner et al. 1997), but also having a powerful impact on the activity of intrinsic biological clock cells (Liu and Reppert 2000, Ehlen et al. 2006). Unique to the SCN, the circadian action of GABA varies due to changes in the intracellular chloride anion concentration (Marcel and Pennartz 2002). It has been shown that GABAergic compounds cause phase shifts in the circadian rhythms (Biggs and Prosser 1998) and alter the biological clock responses to light (Gillespie et al. 1999, Hamada and Shibata 2002, Mintz et al. 2002, Ehlen and Paul 2009) or non-photic stimuli (Gillespie et al. 1997). Accordingly, benzodiazepines ($GABA_A$-targeted drugs commonly used in humans) influence the rhythmic processes of their recipients, having direct impact on the GABAergic structures of the biological clock (Strecker et al. 1999). Interestingly, the phase shifting effect of triazolam is abolished after IGL lesions (Johnson et al. 1988). In case of SCN, it has been found that $GABA_A$ receptors are located postsynaptically on clock cells (Belenky et al. 2003) and modulate the gap junctions (Shinohara et al. 2000), whereas $GABA_B$ receptors are expressed presynaptically (Chen and van den Pol 1998) and modulate the release of other neurotransmitters involved in tuning the circadian activity of SCN cells (Jiang et al. 1995). As yet, very little research has been conducted in the field of GABAergic transmission within the IGL.

Immunohistological studies revealed the presence of $GABA_A$ receptors on GABAergic IGL cells, with different subunit compositions expressed over the soma and the neuropil (Gao et al. 1995). The experiments carried out in our laboratory indicated that blockade of $GABA_A$ receptors by bicuculline or picrotoxin, disrupts the infra-slow oscillation of the IGL neurons *in vivo* (Blasiak and Lewandowski 2004). Moreover, IGL receives GABAergic inputs from such nuclei as the SCN and retrochiasmatic and anterior hypothalamic areas (Vrang et al. 2003).

Therefore, the aim of the study was to evaluate the sensitivity of IGL neurons to one of the most important endogenous neurotransmitters of biological clock. In

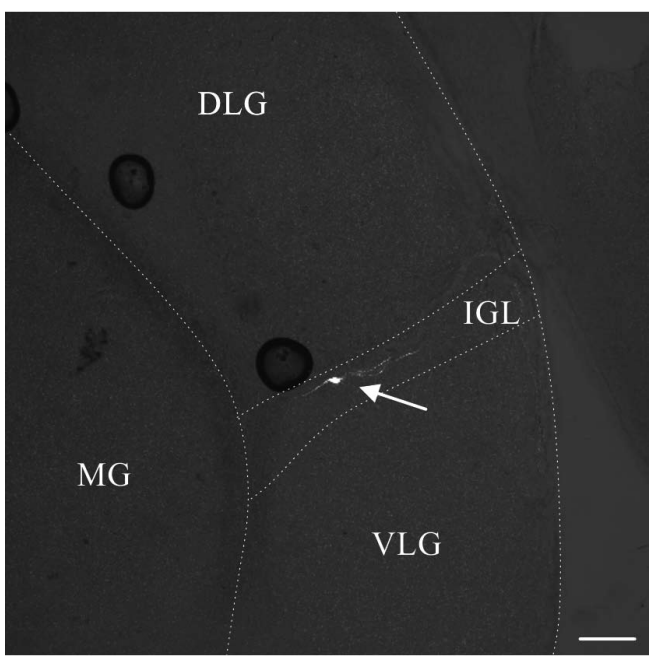

Fig. 1. The intergeniculate leaflet of the thalamus (IGL) localized on the brain slice (100× magnification) with the recorded neuron (white arrow); (DLG) dorsal lateral geniculate nucleus; (VLG) ventral lateral geniculate nucleus; (MG) medial geniculate nucleus. The scale bar is 100 μm.

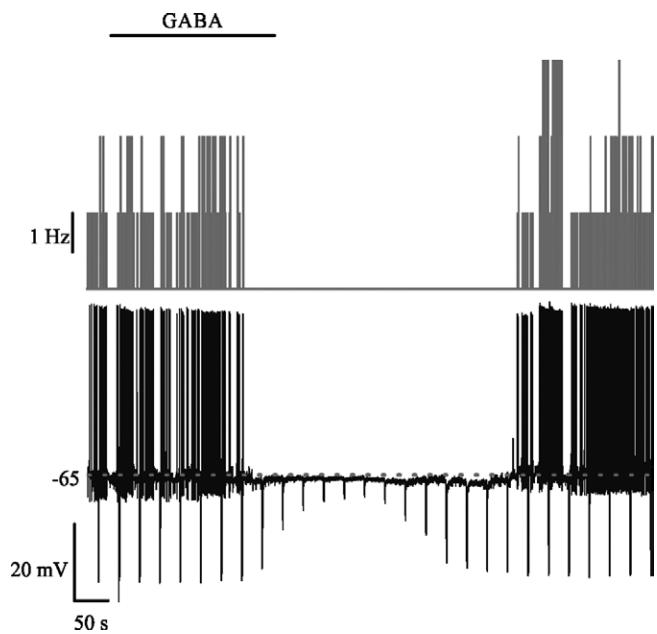

Fig. 2. Representative recording from an IGL neuron. Influence of GABA (100 μM, black bar) on the firing rate in nACSF (above – frequency histogram; below – CC recording). Downwards deflections represent the responses of the IGL cell to a hyperpolarizing current injection (80 pA). About 200 s was needed to substance reach the recording chamber and that is why shift in response is observed.

order to deepen our understanding of IGL physiology, our study focuses on the GABAergic transmission of single IGL neurons and suggests the possible role of both $GABA_A$ and $GABA_B$ receptors. This research not only characterizes the miniature synaptic chloride currents *via* $GABA_A$ receptors, but represents a complex description of the direct electrophysiological effects of GABAergic compounds on the spontaneous neuronal activity of IGL cells *in vitro*.

METHODS

Preparation

All experiments were performed in accordance with the European Community Council Directive of 24 November 1986 (86/0609/EEC) and the Polish Animal Welfare Act of 23 May 2012 (82/2012). They were approved by the Local (Krakow) Ethics Commission. For electrophysiological experiments animals (14 to 18 days old) were held in the 12 h/12 h light/dark conditions (light on 08:00 AM; light off 08:00 PM) with water and food *ad libitum* in Jagiellonian University Animal Facility. Care was taken to minimize the suffering and a number of animals used. For electrophysiological experiments, 12 young male Wistar rats were used. Animals were anesthetized with izoflurane (2 ml/kg body weight, Baxter) and decapitated between 1 and 2 Zeitgeber time (ZT, 09:00–10:00 AM). The brain was quickly removed from the skull and immersed in cold (<4°C) artificial cerebrospinal fluid (ACSF), composed of (in mM): NaCl 118, $NaHCO_3$ 25, KCl 3, NaH_2PO_4 1.2, $CaCl_2$ 2, $MgCl_2$ 2, glucose 10 (pH=7.4; osmolality ~295 mOsmol/kg). Brain slices, 250 µm in thickness, were cut on a Leica vibrotome (VT1000S, Heidelberg, Germany) and then those containing the IGL were placed in a pre-incubation chamber for 60 min at 32°C. Subsequently, each slice with the investigated structure was transferred to the submerged recording chamber and perfused continuously with oxygenated ACSF (95% O_2, 5% CO_2), at room temperature (21°C).

Electrophysiological recordings and data analysis

Using the patch-clamp technique, recordings were obtained from single IGL neurons. All recordings were performed between 4 and 10 ZT using borosilicate glass pipettes (Sutter Instruments, Novato, USA; resistance 6–9 MΩ) that were filled with an internal pipette solution containing, (in mM): potassium gluconate 125, KCl 20, HEPES 10, $MgCl_2$ 2, Na_2ATP 4, Na_3GTP 0.4, EGTA 1 and 0.1% biocytin (pH=7.4 adjusted with 5 M KOH; osmolality ~300 mOsmol/kg; E_{Cl}=−43 mV, E_{K+}=−98 mV). For the recording of miniature inhibitory postsynaptic currents (mIPSC) internal pipette solution with increased KCl concentration (129 mM) instead of potassium gluconate (16 mM) was used to obtain a symmetrical concentration of Cl^- across the membrane. The recording electrode was placed in the IGL region under visual, microscopic control. Recorded neurons were visualized with a 40× objective on a Zeiss Examiner microscope fitted with infrared differential interference contrast (Göttingen, Germany). When desired, a whole-cell configuration was obtained by using negative pressure applied from a Ez-gSEAL100B Pressure Controller (Neo Biosystem, San Jose, USA), and spontaneous activity in current

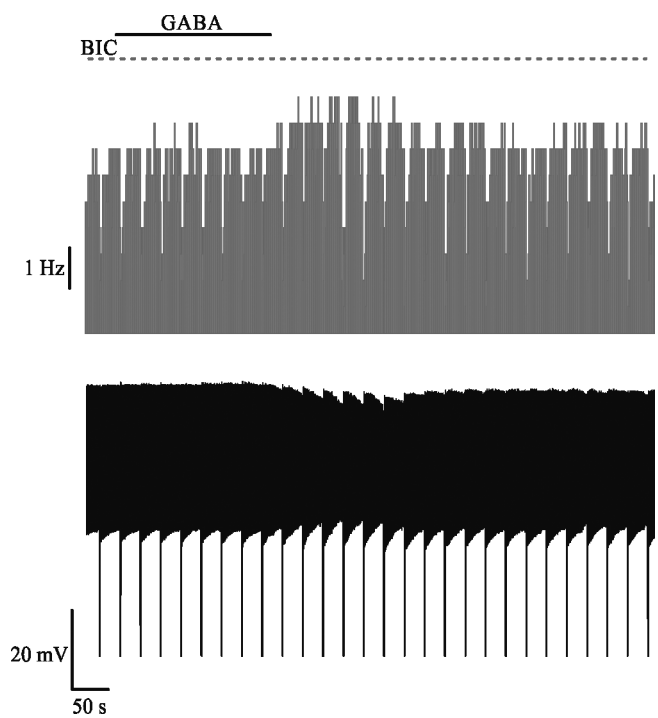

Fig. 3. Representative recording from an IGL neuron. Application of GABA (100 µM) in the presence of bicuculline (Bic, 20 µM, gray bar; above – frequency histogram; below – CC recording). Downwards deflections represent the responses of the IGL cell to a hyperpolarizing current injection (80 pA). About 200 s was needed to substance reach the recording chamber and that is why shift in response is observed.

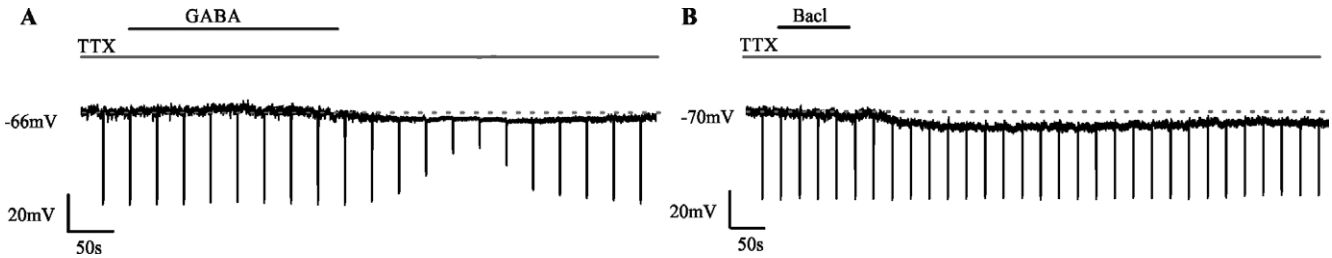

Fig. 4. (A) Representative postsynaptic effect of GABA (100 μM, black bar) in the ACSF containing TTX (0.5 μM, grey bar); (B) effect of baclofen (Bacl, 10 μM, black bar) in the presence of TTX (0.5 μM, grey bar). About 200 s was needed to substance reach the recording chamber and that is why shift in response is observed.

clamp (CC, current: 0 nA) or voltage clamp mode (VC, voltage: −75 mV) was recorded. During each recording in CC, rectangular pulse currents (1 s, 80 pA) were injected to monitor membrane resistance. For recording, Spike2 and Signal software (CED, Cambridge, UK) were used. The recorded signal was amplified by a SC 05LX (NPI, Tamm, Germany) amplifier, digitalized by a Micro1401 mkIII A/D converter (CED) and stored on the computer hard disk. The signal was low-pass filtered at 3 kHz and digitized at 15 kHz. Neurons with a membrane potential more positive than −35 mV (−50 mV when adjusted for the junction potential) were excluded from further analysis.

Analysis was performed in MATLAB (MathWorks, Inc., USA) and Statistica 10.0 (StatSoft, Inc. USA) software. Changes in firing rate or membrane potential were considered to be significant if they differed from baseline by more than three standard deviations (SDs). All data was expressed as mean value ±SEM. The Wilcoxon test and paired *t*-test were used, and $P<0.05$ was considered as significant.

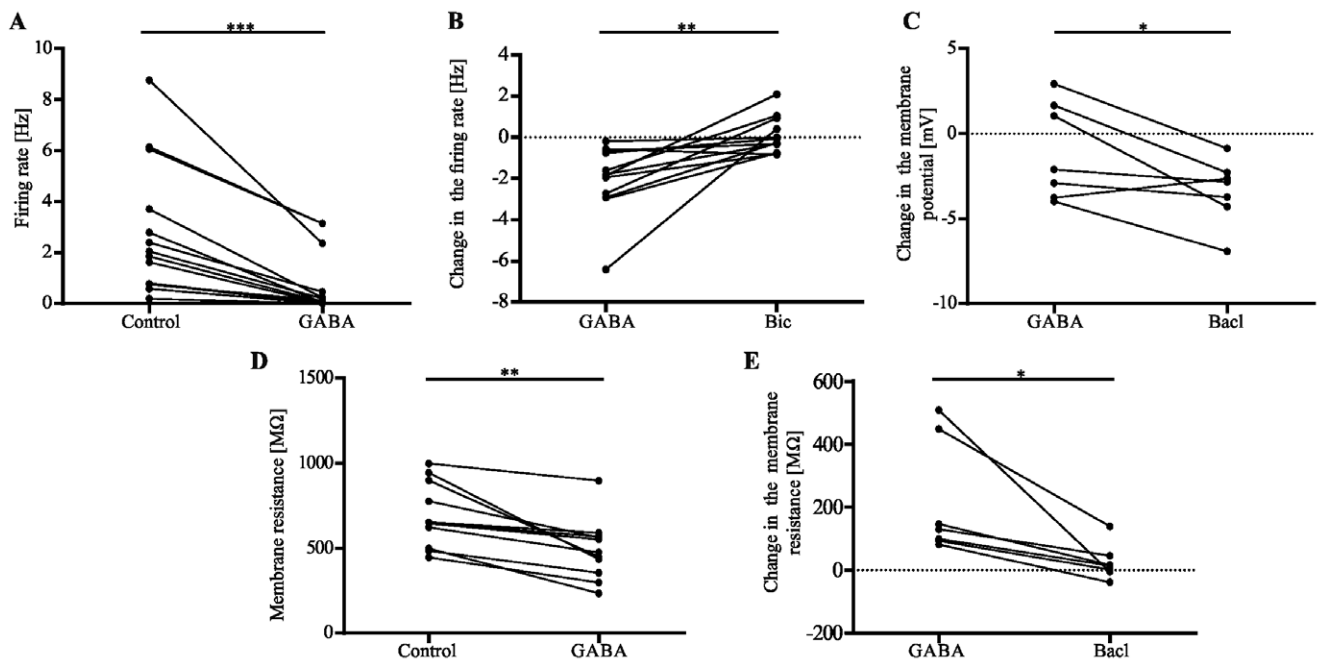

Fig. 5. (A) Change in the firing rate after application of GABA (100 μM, Wilcoxon test, ***$P<0.0001$, $n=14$); (B) comparison of the change in the firing rate caused by GABA application and a second application of GABA in the presence of bicuculline (GABA 100 μM, Bic 20 μM, Wilcoxon test, **$P<0.001$, $n=12$); (C) comparison of change in the membrane potential caused by GABA application and following application of baclofen (GABA 100 μM, Bacl 10 μM, paired *t*-test, *$P<0.05$, $n=7$); (D) Change in the membrane potential after application of GABA (GABA 100 μM, paired *t*-test, **$P<0.001$, $n=11$); (E) comparison of the change in the membrane resistance caused by GABA application and following baclofen application in the presence of TTX (GABA 100 μM, Bacl 10 μM , TTX 0.5 μM, Wilcoxon test, *$P<0.05$, $n=7$).

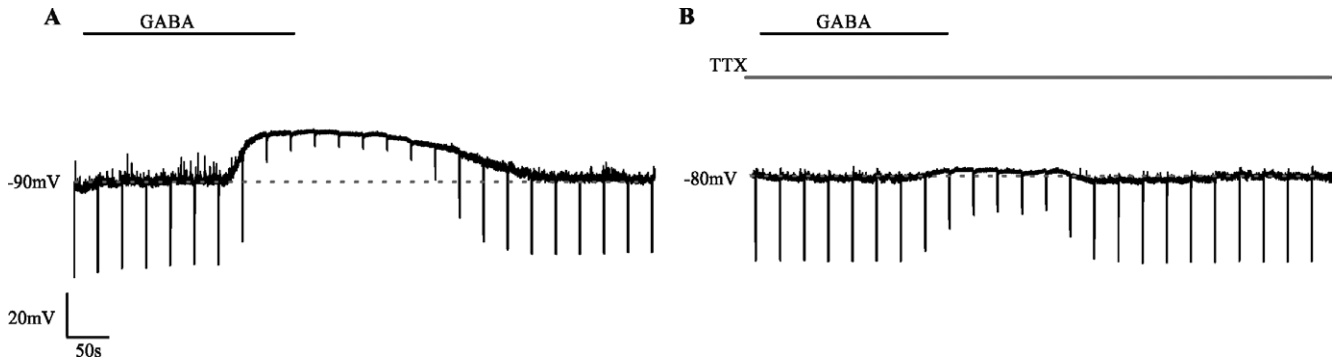

Fig. 6. The depolarization of an IGL neuron by GABA (100 μM, black bar) application. (A) The change in membrane potential of the silent neuron in nACSF after application of the drug; (B) the membrane potential of an IGL neuron after application of the drug in ACSF enriched in TTX (0.5 μM). About 200 s was needed to substance reach the recording chamber and that is why shift in response is observed.

Immunohistochemistry

At the end of each electrophysiological experiment, slices with the tested cell were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) at 4°C over night. After that, the slices were rinsed in PBS (2×10 min) and subsequently placed in a 0.6% solution of Triton X-100 in PBS for 24 h. In order to visualize cells filled with biocytin the slices were incubated in PBS containing ExtrAvidyn conjugated Cy3 (1:250). At the end, the slices were rinsed again in PBS and mounted on gelatine-coated glass slides with glycerol (Sigma-Aldrich). After completion of the histological procedure, the slices were viewed under an Axiovert 200 confocal microscope equipped with LSM 510 META confocal module (Zeiss, Jena, Germany). The images were further analyzed with AxioVision (Zeiss) software.

Reagents

Stock solutions of: GABA (Sigma-Aldrich), Bicuculline (Bic, Tocris, Bristol, UK), Baclofen (Bacl, Sigma-Aldrich), Kynurenic acid sodium salt (Kyn, Tocris, Bristol, UK) and tetrodotoxin (TTX, Tocris, Bristol, UK) were prepared in distilled water (100× concentrated) and kept at −20°C. Working solutions: GABA 100 μM, Bic 20 μM, Bacl 10 μM, Kyn 20 μM and TTX 0.5 μM were prepared fresh in ACSF on the day of experiment. All drugs were delivered by bath perfusion and ~200 s was needed for the substance to reach the recording chamber.

RESULTS

The IGL neurons were identified as a group of neurons oriented perpendicularly to the long axis of IGL from the young pups (Fig. 1). In total 34 neu-

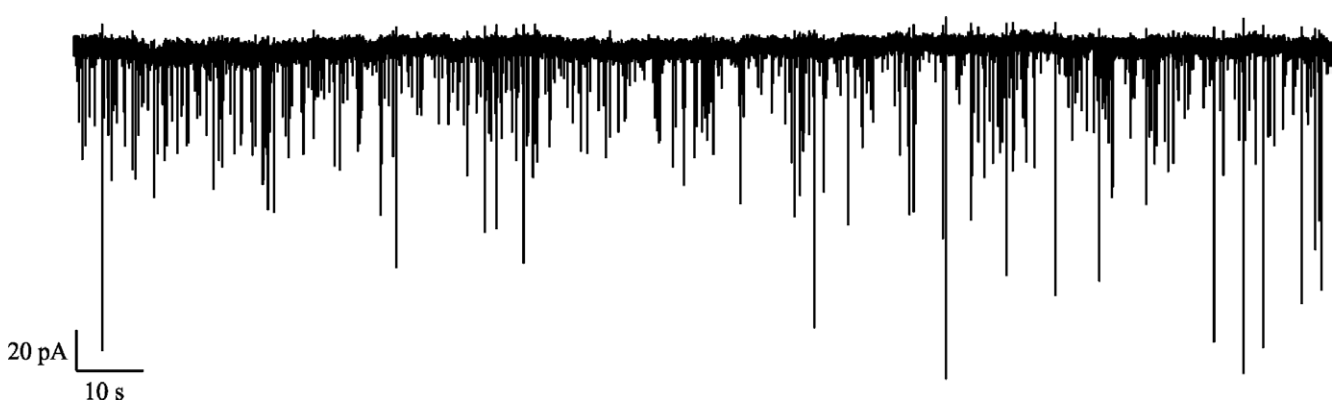

Fig. 7. Representative VC recording (holding voltage: −75 mV) with mIPSC in the presence of TTX (0.5 μM) and kynurenic acid sodium salt (20 μM).

rons were recorded: 19 and 15 in CC or VC mode, respectively. In the current clamp mode most of the IGL neurons were spontaneously active, with a mean firing rate of 2.74±0.68 Hz (*n*=14). A minority of neurons were silent in the normal ACSF (nACSF), with a mean membrane potential of −74.35±3.07 mV (*n*=5). GABA application (100 μM) caused a significant decrease in firing rate in all spontaneously active neurons, with a mean change −2.04±0.43 Hz (from 2.74±0.68 Hz to 0.75±0.32 Hz, *n*=14, Figs 2 and 5A) and hyperpolarization in silent neurons with a mean change −2.09±1.26 mV (from −71.42±4.52 mV to −74.90±4.07 mV, *n*=3, data not shown) or depolarization with a mean change 9.40±6.70 mV (from −78.74±1.04 mV to −69.34±5.95 mV, *n*=2, Fig. 6A). In order to evaluate whether the effects of GABA were pre- or postsynaptic, we added TTX (0.5 μM) to the nACSF, which blocked voltage gated sodium channels. Under these conditions, GABA caused hyperpolarization with a mean change of −3.24±0.43 mV (from −61.15±3.32 mV to −64.39±3.38 mV, *n*=8, Fig. 4A) and in three cells GABA caused depolarization, with a mean change of 1.88±0.55 mV (from −64.08±4.02 mV to −62.55±4.73 mV, *n*=3, Fig. 6B). In both cases a significant decrease in input resistance, with a mean change of 215±45 MΩ (from 692±57MΩ to 492±53 MΩ, *n*=11, Fig. 5D) was observed.

Our next question was which GABA receptor is involved in the response to GABA. In the case of 12 neurons from a total of 19 recorded in CC mode, we checked if the effect of GABA was blocked by a specific $GABA_A$ antagonist – bicuculline. In the presence of bicuculline (20 μM), GABA (100 μM) was applied again and we observed the small increase in firing rate with a mean change of 0.09±0.26 Hz (from 3.05±0.81 Hz to 3.14±0.89 Hz, *n*=12, Figs 3 and 5B). This effect was not accompanied by a statistically significant change in the input resistance (data not shown). As a next step we checked if $GABA_B$ receptors are involved in the observed responses. In the case of 7 neurons we applied baclofen – a specific agonist of $GABA_B$ receptors, in the presence of TTX in ACSF. Hyperpolarization was observed, with a mean change of −4.17±0.87 mV (from −65.89±2.36 mV to −70.06±2.68 mV, *n*=7, Figs 4B and 5C) after baclofen application. A statistically significant decrease in input resistance, with a mean of change of 25±21 MΩ (*n*=7, Fig. 5E), was also observed.

We also performed recordings in VC mode in order to evaluate miniature IPSCs (mIPSCs). We used ACSF with additional TTX and kynurenic acid, to isolate recorded neurons and to block glutamatergic transmission. Intrapipette solution was enriched in chloride ions (see section Methods). mIPSCs were present in all recorded cells (*n*=15, Fig. 7), the mean amplitude was 42±3 pA and the mean frequency was 1.02±0.1 Hz. To check if there are any distinguishable groups of GABAergic neurons in the IGL based on mIPSC characteristics, we plotted mIPSC mean amplitude against mean frequency. In neither case, for either parameters of mIPSC, did the recorded neurons form identifiable clusters (Fig. 8).

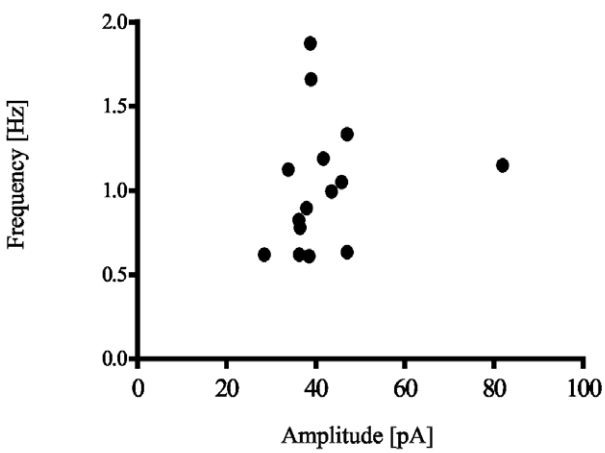

Fig. 8. Mean values of amplitude and frequency of mIPSCs plotted against each other. Each dot represents a measurement from one the IGL neuron.

DISCUSSION

In this study we have shown the variability in the neuronal responses of single IGL neurons to GABAergic compounds. We have found that application of GABA (100 μM) significantly decreased the input resistance of IGL neurons which suggests its main action is *via* the ionotropic $GABA_A$ receptor. Because of the evoked chloride current, a decrease in the firing frequency was the accompanying effect observed in all spontaneously active neurons (*n*=14). This effect was dose dependent. The observed effects of GABA on the membrane potential were, however, not identical in all the tested neurons, both the silent neurons in nACSF and the cells tested in the presence of TTX (0.5 μM) in

ACSF. Cumulatively, 11 cells were hyperpolarized and 2 were depolarized by GABA. This discrepancy may be explained by the mechanism of GABA action on two different GABAergic receptors ($GABA_A$ and $GABA_B$) and their distinct expression on the cellular membrane of IGL cells. A higher proportion of $GABA_A$ receptors should act to shift the membrane potential towards the reversal potential of the chloride ions (−43 mV in our research), which causes depolarization. In contrast the $GABA_B$ receptors, functionally coupled mainly with the potassium channels, which reversal potential in our conditions was −98 mV, can explain hyperpolarization. The other possible factor is the variability of resting membrane potentials of the recorded cells (which are not only dependent on the chloride ions), which can be changed by GABA to either more depolarized or hyperpolarized values.

Membrane potential depolarization by an inhibiting neurotransmitter has been previously described in the developing brain. In adult, under physiological conditions, SCN neurons can perform excitatory responses after GABA application during the day (Wagner et al. 1997). This activation is dependent on the chloride co-transporter (sodium-potassium-chloride co-transporter, NKCC) and subsequent transient calcium current (Choi et al. 2008). The probable mechanism suggests that the high concentration of chloride ions in the neuronal cytoplasm caused by NKCC activation leads to reversal of the ionic current and therefore depolarization after GABA binding to the $GABA_A$ receptor. Another study highlights the role of L-type calcium channels (Irwin and Allen 2009). In our whole-cell patch clamp research, the concentration of chloride ions inside the recorded neuron was set by the intra-pipette solution. That is why, accumulation of chloride anions caused by active NKCC seems unlikely. However, we are not able to exclude a fundamental role for calcium currents acting *via* L-type channels in the observed depolarization.

To check the possible role of $GABA_B$ receptors in modulating the spontaneous activity of IGL neurons, we applied GABA (100 μM) in the presence of a $GABA_A$ receptor blocker – bicuculline (20 μM, n=13). Although the changes evoked were not statistically significant, either in terms of the firing rate or the input resistance, we could observe slight perturbations in neuronal activity – an increase or decrease in the firing rate. The probable explanation for this variability in the responses stems from two opposing factors influencing membrane potential – membrane hyperpolarization *via* $GABA_B$ receptors on the recorded cell vs. network disinhibition caused by the influence of GABA on other IGL GABAergic interneurons preserved in the brain slice. Based on this rationale, we used a specific $GABA_B$ receptor agonist – baclofen (10 μM), in the presence of TTX (0.5 μM) in ACSF (n=7) to exclude the impact of neighbouring neurons. For the first time, we have shown the hyperpolarizing effect of GABA compounds on IGL cells *via* $GABA_B$ receptors and therefore we suggest the expression of those receptors by IGL neurons. The change in the membrane potential was accompanied by a decrease in the input resistance, probably deriving from the enhancement of potassium conductance. Our findings, regarding the role of both $GABA_A$ and $GABA_B$ receptors in IGL neuron inhibition, could be compared to related brain structures in which GABA exerts similar effects through the same pathways. Indeed, both $GABA_A$ and $GABA_B$ receptors in SCN play different roles in circadian timing system physiology (Jiang et al. 1995, Chen and van den Pol 1998, Shinohara et al. 2000, Belenky et al. 2003). Moreover, the dorsal and ventral part of lateral geniculate nucleus, the neighbouring and interrelated nuclei, are known to be differentially controlled *via* $GABA_A$ and $GABA_B$ receptor pathways (Soltesz et al. 1989, Zhu and Lo 1999, Bright et al. 2007).

To supplement our research into GABAergic modulation of IGL neurons, we conducted experiments in voltage clamp mode in order to measure the GABAergic spontaneous synaptic activity. The presence of mIPSCs indicates that all the recorded neurons (n=15) were innervated by GABAergic cells. Moreover, we could not distinguish any groups based on different amplitudes or frequency of mIPSC. This result emphasizes the leading role of GABA as the main neurotransmitter of time keeping neuronal structures, although dense GABAergic projections coinciding with high frequency mIPSCs are common in many brain structures (Kusek et al. 2010, Szczot et al. 2010). The synaptic inputs may come from the surrounding IGL neurons as well as from more remote neuronal populations (Vrang et al. 2003).

CONCLUSIONS

The important role of GABA in the physiology of IGL was previously a topic of research in our laboratory (Blasiak and Lewandowski 2004). However, the *in vivo* studies indicating that GABAergic transmis-

sion takes part in generating the ISO pattern of neuronal activity could not answer our question regarding the direct effects of GABA on IGL neurons. It is worth mentioning here that the research into serotonergic modulation of IGL by dorsal raphe GABAergic neurons (Blasiak et al. 2006) cannot exclude the role of GABA in the observed effect of ISO disruption after stimulation of dorsal raphe *in vivo*. The present research is also an important addition to our latest *in vitro* study concerning the direct depolarizing effect of serotonin on single IGL neurons (Palus et al. 2013), and can partially explain discrepancies between the opposing effects of this neurotransmitter under different conditions. Therefore, the probable role of GABA in the IGL neuronal network is highly complex, and further research clarifying the interactions between GABA and other neurotransmitters conveying photic and non-photic information is certainly needed.

ACKNOWLEDGEMENTS

The authors would like to thank Professor Bogdan Dreher from University of Sydney, for his comments and language correction of the final version of manuscript. Supported by the K/DSC/002339 obtained from the Institute of Zoology of the Jagiellonian University.

REFRENCES

Belenky MA, Sagiv N, Fritschy JM, Yarom Y (2003) Presynaptic and postsynaptic $GABA_A$ receptors in rat suprachiasmatic nucleus. Neuroscience 118: 909–923.

Biggs KR, Prosser RA (1998) $GABA_B$ receptor stimulation phase-shifts the mammalian circadian clock in vitro. Brain Res 807: 250–254.

Blasiak T, Lewandowski MH (2004) Blockade of $GABA_A$ receptors disrupts isoperiodic neuronal oscillations in the intergeniculate leaflet of the rat. Brain Res 1009: 82–87.

Blasiak T, Lewandowski MH (2013) Differential firing pattern and response to lighting conditions of rat intergeniculate leaflet neurons projecting to suprachiasmatic nucleus or contralateral intergeniculate leaflet. Neuroscience 228: 315–324.

Blasiak T, Siejka S, Raison S, Pevet P, Lewandowski MH (2006) The serotonergic inhibition of slowly bursting cells in the intergeniculate leaflet of the rat. Eur J Neurosci 24: 2769–2780.

Bormann J, Feigenspan A (1995) $GABA_C$ receptors. Trends Neurosci 18: 515–519.

Bright DP, Aller MI, Brickley SG (2007) Synaptic release generates a tonic $GABA_{(A)}$ receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J Neurosci 27: 2560–2569.

Card JP, Moore RY (1989) Organization of lateral geniculate-hypothalamic connections in the rat. J Comp Neurol 284: 135–147.

Chen G, van den Pol AN (1996) Multiple NPY receptors coexist in pre- and postsynaptic sites: inhibition of GABA release in isolated self-innervating SCN neurons. J Neurosci 16: 7711–7724.

Chen G, van den Pol AN (1998) Presynaptic $GABA_B$ autoreceptor modulation of P/Q-type calcium channels and GABA release in rat suprachiasmatic nucleus neurons. J Neurosci 18: 1913–1922.

Choi HJ, Lee CJ, Schroeder A, Kim YS, Jung SH, Kim JS, Kim do Y, Son EJ, Han HC, Hong SK, Colwell CS, Kim YI (2008) Excitatory actions of GABA in the suprachiasmatic nucleus. J Neurosci 28: 5450–5459.

Ehlen JC, Paul KN (2009) Regulation of light's action in the mammalian circadian clock: role of the extrasynaptic $GABA_A$ receptor. Am J Physiol Regul Integr Comp Physiol 296: 1606–1612.

Ehlen JC, Novak CM, Karom MC, Gamble KL, Paul KN, Albers HE (2006) $GABA_A$ receptor activation suppresses Period 1 mRNA and Period 2 mRNA in the suprachiasmatic nucleus during the mid-subjective day. Eur J Neurosci 23: 3328–3336.

Gao B, Fritschy JM, Moore RY (1995) $GABA_A$-receptor subunit composition in the circadian timing system. Brain Res 700: 142–156.

Gillespie CF, Mintz EM, Marvel CL, Huhman KL, Albers HE (1997) GABA and GABA agonists and antagonists alter the phase-shifting effects of light when microinjected into the suprachiasmatic region Brain Res 759: 181–189.

Gillespie CF, van der Beek EM, Mintz EM, Mickley NC, Jasnow M, Huhman KL, Albers HE (1999) GABAergic regulation of light-induced c-Fos immunoreactivity within the suprachiasmatic nucleus. J Comp Neurol 411: 683–692.

Hamada T, Shibata S (2002) The role of GABAergic neuron on NMDA- and SP-induced phase delays in the suprachiasmatic nucleus neuronal activity rhythm *in vitro*. Neurosci Lett 468: 344–347.

Harrington ME (1997) The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. Neurosci Biobehav Rev 21: 705–727.

Irwin RP, Allen CN (2009) GABAergic signaling induces divergentneuronal Ca2+ responses in the suprachiasmatic nucleus network. Eur J Neurosci 30: 1462–1475.

Jacob N, Vuillez P, Lakdhar-Ghazal N, Pévet P (1999) Does the intergeniculate leaflet play a role in the integration of the photoperiod by the suprachiasmatic nucleus? Brain Res 828: 83–90.

Jiang ZG, Allen CN, North RA (1995) Presynaptic inhibition by baclofen of retinohypothalamic excitatory synaptic transmission in rat suprachiasmatic nucleus. Neuroscience 64: 813–819.

Johnson RF, Smale L, Moore RY, Morin LP (1988) Lateral geniculate lesions block circadian phase-shift responses to a benzodiazepine. Proc Natl Acad Sci U S A 85: 5301–5304.

Juhl F, Hannibal J, Fahrenkrug J (2007) Photic induction of c-Fos in enkephalin neurons of the rat intergeniculate leaflet innervated by retinal PACAP fibres. Cell Tissue Res 329: 491–502.

Kusek M, Tokarski K, Hess G (2010) Repeated restraint stress enhances glutamatergic transmission in the paraventricular nucleus of the rat hypothalamus. J Physiol Pharmacol 64: 565–570.

Lall GS, Biello SM (2003) Neuropeptide Y, GABA and circadian phase shifts to photic stimuli. Neuroscience 120: 915–921.

Lewandowski MH, Usarek A (2002) Effects of intergeniculate leaflet lesions on circadian rhythms in the mouse. Behav Brain Res 128: 13–17.

Liu C, Reppert SM (2000) GABA synchronizes clock cells within the suprachiasmatic circadian clock. Neuron 25: 123–128.

Marcel DJ, Pennartz C (2002) Circadian modulation of GABA function in the rat suprachiasmatic nucleus: excitatory effects during the night phase. J Neurophysiol 87: 834–844.

Mintz EM, Jasnow M, Gillespie CF, Huhman KL, Albers HE (2002) GABA interacts with photic signaling in the suprachiasmatic nucleus to regulate circadian phase shifts. Neuroscience 109: 773–778.

Moore RY, Card JP (1994) Intergeniculate leaflet: an anatomically and functionally distinct subdivision of the lateral geniculate complex. J Comp Neurol 344: 403–430.

Moore RY, Speh JC (1993) GABA is the principial neurotransmitter of the circadian system. Neurosci Lett 150: 112–116.

Palus K, Chrobok L, Lewandowski MH (2013) Depolarization of the intergeniculate leaflet neurons by serotonin - in vitro study. J Physiol Pharmacol 64: 773–778.

Roberts E (2007) Gamma-aminobutyric acid. Scholarpedia 2: 3356.

Saderi N, Cazarez-Márquez F, Buijs FN, Salgado-Delgado RC, Guzman-Ruiz MA, del Carmen Basualdo M, Escobar C, Buijs RM (2013) The NPY intergeniculate leaflet projections to the suprachiasmatic nucleus transmit metabolic conditions. Neuroscience 246: 291–300.

Shinohara K, Hiruma H, Funabashi T, Kimura F (2000) GABAergic modulation of gap junction communication in slice cultures of the rat suprachiasmatic nucleus. Neuroscience 96: 591–596.

Soltesz I, Lightowler S, Leresche N, Crunelli V (1989) Optic tract stimulation evokes $GABA_A$ but not $GABA_B$ IPSPs in the rat ventral lateral geniculate nucleus. Brain Res 479: 49–55.

Strecker GJ, Park WK, Dudek FE (1999) Zinc and flunitrazepam modulation of GABA-mediated currents in rat suprachiasmatic neurons. J Neurophysiol 81: 184–191.

Szczot M, Wojtowicz T, Mozrzymas JW (2010) GABAergic and glutamatergic currents in hippocampal slices and neuronal cultures show profound differences: a clue to a potent homeostatic modulation. J Physiol Pharmacol 61: 501–506.

Takatsuji K, Tohyama M (1989) Geniculo-geniculate projection of enkephalin and neuropeptide Y containing neurons in the intergeniculate leaflet of the thalamus in the rat. J Chem Neuroanat 2: 19–27.

Vrang N, Mrosovsky N, Mikkelsen JD (2003) Afferent projections to the hamster intergeniculate leaflet demonstrated by retrograde and anterograde tracing. Brain Res Bull 59: 267–288.

Wagner S, Castel M, Gainer H, Yarom Y (1997) GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. Nature 387: 598–603.

Zhu JJ, Lo FS (1999) Three GABA receptor-mediated postsynaptic potentials in interneurons in the rat lateral geniculate nucleus. J Neurosci 19: 5721–5730.