Journal of Biomedical Optics

BiomedicalOptics.SPIEDigitalLibrary.org

# On involvement of transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells, activator protein-1 and signal transducer and activator of transcription-3 in photodynamic therapy-induced death of crayfish neurons and satellite glial cells

Elena Berezhnaya
Marya Neginskaya
Vera Kovaleva
Svetlana Sharifulina
Irina Ischenko
Maxim Komandirov
Mikhail Rudkovskii
Anatoly B. Uzdensky

SPIE.

# On involvement of transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells, activator protein-1 and signal transducer and activator of transcription-3 in photodynamic therapy-induced death of crayfish neurons and satellite glial cells

**Elena Berezhnaya, Marya Neginskaya, Vera Kovaleva, Svetlana Sharifulina, Irina Ischenko, Maxim Komandirov, Mikhail Rudkovskii, and Anatoly B. Uzdensky***
Southern Federal University, Academy of Biology and Biotechnology, 194/1 Stachky Prospect, Rostov-on-Don 344090, Russia

**Abstract.** Photodynamic therapy (PDT) is currently used in the treatment of brain tumors. However, not only malignant cells but also neighboring normal neurons and glial cells are damaged during PDT. In order to study the potential role of transcription factors—nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein (AP-1), and signal transducer and activator of transcription-3 (STAT-3)—in photodynamic injury of normal neurons and glia, we photosensitized the isolated crayfish mechanoreceptor consisting of a single sensory neuron enveloped by glial cells. Application of different inhibitors and activators showed that transcription factors NF-κB (inhibitors caffeic acid phenethyl ester and parthenolide, activator betulinic acid), AP-1 (inhibitor SR11302), and STAT-3 (inhibitors stattic and cucurbitacine) influenced PDT-induced death and survival of neurons and glial cells in different ways. These experiments indicated involvement of NF-κB in PDT-induced necrosis of neurons and apoptosis of glial cells. However, in glial cells, it played the antinecrotic role. AP-1 was not involved in PDT-induced necrosis of neurons and glia, but mediated glial apoptosis. STAT-3 was involved in PDT-induced apoptosis of glial cells and necrosis of neurons and glia. Therefore, signaling pathways that regulate cell death and survival in neurons and glial cells are different. Using various inhibitors or activators of transcription factors, one can differently influence the sensitivity and resistance of neurons and glial cells to PDT.  [DOI: 10.1117/1.JBO.20.7.075004]

Keywords: photodynamic therapy; transcription factors; cell death; neuron; glial cell.

Paper 150178R received Mar. 18, 2015; accepted for publication Jun. 8, 2015; published online Jul. 9, 2015.

## 1 Introduction

In photodynamic therapy (PDT), the energy of photoexcitation is transferred from the photosensitizer molecules, which stain cells and tissues, to oxygen and transforms it to a highly toxic singlet state. This leads to oxidative stress and cell death.[1–3] PDT is currently used in neuro-oncology for the treatment of brain tumors.[4–9] However, not only malignant cells but also neighboring normal neuronal and glial cells are damaged during PDT. Therefore, it is of importance to study the mechanisms of photodynamic injury of normal neurons and glia.

PDT-induced cell death is controlled by the complex signal transduction system.[2,3,10–12] Signal proteins successively activate each other and different executive proteins by covalent modifications, such as phosphorylation or limited proteolysis. The next regulation level is initiated when the number of present signaling and executive proteins is insufficient. In this case, transcription factors initiate gene expression and the production of additional protein molecules.

Transcription factors—nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB),[13–16] activator protein 1 (AP-1),[17] and signal transducer and activator of transcription-3 (STAT-3)[18]—regulate various physiological processes such as cell responses to environmental stimuli including oxidative stress, differentiation, proliferation, or apoptosis. NF-κB is known as a redox-activated transcription factor.[15,16] In various cells including neurons, it is inactive and does not require new protein synthesis for activation.[14] AP-1 is a dimer composed of subunits belonging to the Jun and Fos families. Photodynamic treatment has been reported to activate NF-κB[19–23] and AP-1[24,25] and stimulate their translocation into the nucleus, where they initiate gene expression. STAT-3 is a component of the Janus kinase (Jak)/STAT signaling pathway. Upon the binding of growth factors to their receptors, Jak phosphorylates its own and receptor's tyrosines. After recruitment by phosphotyrosines, STAT-3 is phosphorylated and forms dimers, which are translocated into the nucleus and regulate gene expression.[26] However, the role of these transcription factors in PDT-induced injury of nerve and glial cells has not been studied yet.

In the mammalian brain, numerous neurons are interconnected with hundreds of other neurons. The number of glial cells is 10 times higher than the number of neurons. It is very difficult to identify neuronal and glial cells in the brain and study the processes of interest in individual neurons and glial cells.

*Address for correspondence: Anatoly B. Uzdensky, E-mail: auzd@yandex.ru

1083-3668/2015/$25.00 © 2015 SPIE

Such studies provide usually averaged results. It is reasonable to study the simpler nervous systems of invertebrates. In order to explore the involvement of various transcription factors in PDT-induced death of neurons and glia, we used a simple but informative model object—isolated crayfish stretch receptor, in which a single mechanoreceptor neuron (MRN) is surrounded by satellite glial cells so that the neuroglial interactions are exactly indentified.[27] This neuron maintains regular firing during 8 to 10 h. PDT inactivates this neuron and induces its necrosis as well as necrosis and apoptosis of surrounding glial cells. The involvement of diverse signaling processes in responses of this neuron and glial cells to photodynamic impact has been thoroughly studied.[28–33]

In the present study, we used various inhibitors and activators of NF-κB, AP-1, and STAT-3 to explore their potential involvement in PDT-induced inactivation, necrosis, and apoptosis of neurons and glial cells.

## 2 Materials and Methods

The activator of NF-κB BA, NF-κB inhibitor caffeic acid phenethyl ester (CAPE), AP-1 inhibitor SR11302, STAT-3 inhibitors stattic (6-Nitrobenzo[b]thiophene-1,1-dioxide) and cucurbitacin I hydrate, fluorochromes propidium iodide and Hoechst 33342 was supplied by Sigma-Aldrich-Rus (Moscow). Another NF-κB inhibitor, parthenolide (sesquiterpene lactone), was obtained from Alomone Labs (Israel). The photosensitizer Photosens, a mixture of sulphonated aluminum phthalocyanines, $AlPcS_n$ (mean $n = 3.1$) was produced by the institute NIOPIK (Moscow, Russia).

The abdominal stretch receptors of the crayfish *Astacus leptodactylus* (Fig. 1) were isolated as described previously.[28,29] The crayfishes from Don river were purchased on the local market. The animal care protocol corresponded to the institutional guidelines in the Academy of Biology and Biotechnology of the Southern Federal University. All experimental procedures were performed according to the European Union guidelines 86/609/EEC for the use of experimental animals and local legislation for ethics of experiments on animals. The isolated stretch receptors were placed into a plexiglass chamber equipped with a device for receptor muscle extension and filled with 2 ml of van Harreveld saline (mM: NaCl—205, KCl—5.4, $NaHCO_3$—0.2, $CaCl_2$—13.5, $MgCl_2$—5.4, pH 7.2 to 7.4). Neuron spikes were recorded extracellularly from axons by the glass pipette suction electrodes, amplified, digitized by the analog–digital converter L-761 (L-Card, Moscow, Russia), and processed by a personal computer using home-made software that provided continuous monitoring of firing. Experiments were carried out at $24 \pm 4$°C.

At the beginning of the experiment, the initial level of neuronal activity was set near 6 to 10 Hz by application of the appropriate receptor muscle extension. After the recording of 30-min firing, sulphonated alumophthalocyanine Photosens (50 nM) and the modulator of a studied transcription factor were added into the chamber with an interval of 3 to 5 min. Following 30-min incubation, cells were irradiated for 30 min with the diode laser (NII "Polus," Moscow, Russia; 670 nm, 0.4 $W/cm^2$). This regime of photodynamic treatment of the isolated crayfish stretch receptor has been used in our previous studies.[31–33] The concentrations of drugs used for inhibitor/activator analysis were determined in the preliminary experiments so that the durations of MRN firing in dark were about 2 to 3 h (Table 1), i.e., these drugs influenced the neuron but did not disturb its activity so strongly as the photodynamic effect (10 to 30 min). The halves of these concentrations were used in further experiments.

In order to visualize dead neurons and glial cells, 20-$\mu$M propidium iodide and 10 to 20 $\mu$M Hoechst 33342 were added into the experimental chamber 8 h after the irradiation (the interval necessary for apoptosis development). Then the preparations were washed with saline, fixed with 0.2% glutaraldehyde, repeatedly washed, and mounted in glycerol. Fluorescent images were acquired using the fluorescence microscopes Lumam-I3 (LOMO, Sankt-Petersburg, Russia) or Nikon Eclipse FN1 (Japan). Propidium iodide, a membrane impermeable fluorochrome, imparts red fluorescence to nuclei of necrotic cells with a compromised plasma membrane. Hoechst 33342 imparts blue fluorescence to the nuclear chromatin. It visualizes intact nuclei of living cells and fragmented nuclei of apoptotic cells (Fig. 2). Red nuclei of necrotic cells stained by propidium iodide were counted in the predetermined standard field ($100\ \mu m \times 100\ \mu m$) around the soma of the sensory receptor neuron so that the neuron nucleus was situated in its center. Fragmented nuclei of apoptotic glial cells were counted around the proximal 2-mm axon fragment where glial apoptosis was most profound. One way analysis

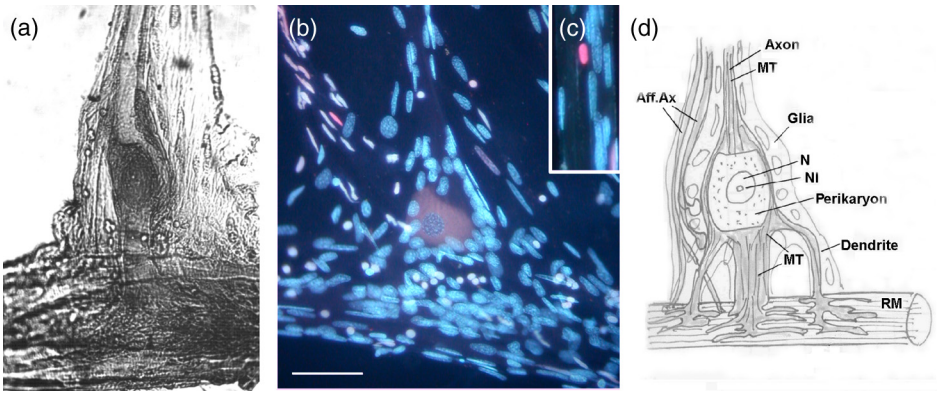


**Fig. 1** Slowly adapting abdominal stretch receptor of a crayfish. (a) Brightfield microphotograph. Fluorescent images of the stretch receptor (b) and the fragment of its axon (c) fluorochromed with Hoechst 33342 (blue fluorescence of the cellular nuclei) and propidium iodide (red fluorescence of nuclei of necrotic cells). (d) Scheme of this preparation. Mechanoreceptor neuron surrounded by glial cells is attached to the receptor muscle (RM). Its axon and dendrites are filled with numerous microtubules (MT). Dendrite endings are ramified between muscle fibers and contact with their membranes. N, nucleus; Nl, nucleolus; Aff.Ax, afferent axons. Scale bar 50 $\mu$m (adapted from Ref. 27).

**Table 1** The effects of the drugs used in the present paper on duration of firing of isolated crayfish stretch receptor in darkness. The drug concentrations further used in the experiments are bold. Mean ± standard error of the mean.

| Substance | Concentration ($\mu$M) | Firing duration (min) |
|---|---|---|
| Betulinic acid | 2 | 359 ± 54 |
| | **5** | 179 ± 38 |
| | 10 | 122 ± 27 |
| SR11302 | 2.5 | 248 ± 33 |
| | 5 | 195 ± 35 |
| | **10** | 116 ± 27 |
| CAPE | 10 | 446 ± 21 |
| | **30** | 202 ± 36 |
| Parthenolide | 10 | 382 ± 52 |
| | **20** | 234 ± 43 |
| | 50 | 113 ± 28 |
| Stattic | 0.2 | 320 ± 34 |
| | 0.5 | 217 ± 41 |
| | **1** | 142 ± 18 |
| | 2 | 95 ± 27 |
| Cucurbitacin | 0.01 | 364 ± 63 |
| | **0.05** | 218 ± 28 |
| | 0.1 | 197 ± 79 |
| | 0.2 | 130 ± 26 |
| | 0.5 | 157 ± 17 |
| | 1 | 123 ± 39 |

Note: CAPE, caffeic acid phenethyl ester.

of variance (ANOVA) was used for statistical evaluation of the difference between experimental groups. Data are presented as Mean ± standard error of the mean.

## 3 Results

After stretch receptor isolation, the MRN fired 6 to 8 h in darkness until irreversible cessation of its activity. Necrosis of isolated MRN and satellite glial cells and glial apoptosis at that time were insignificant [Figs. 3(a)–8(a)]. The nuclear fragmentation characteristic for apoptosis was never observed in MRN.[28–33] Possibly, as in many adult vertebrate neurons,[34] apoptosis of MRN is intrinsically blocked[28,29] in order to prevent an occasional loss of functions important for the whole organism.

As in previous experiments,[28–33] neither Photosens at a concentration <10 $\mu$M nor laser radiation changed neuronal activity and survival by themselves. However, their combined action, i.e., PDT markedly shortened the duration of neuronal activity

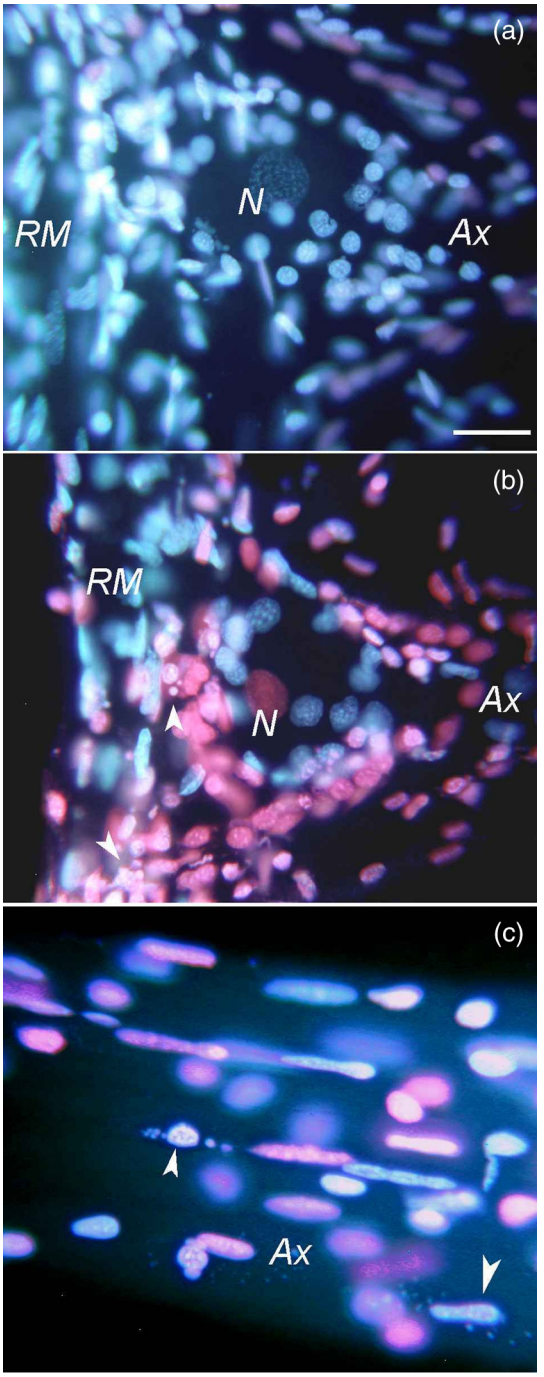


**Fig. 2** Photodynamic theraphy-induced death of neurons and glial cells in the crayfish stretch receptor. (a) Fluorescence of the control preparation. Nuclei of living cells are blue; (b) fluorescence of the photosensitized preparation. Necrotic nuclei of the mechanoreceptor neuron (N) and satellite glial cells stained with propidium iodide fluoresce in red; (c) the fragment of axon (Ax) of the photosensitized neuron surrounded by glial cells. Fragmented glial nuclei (arrowheads) belong to apoptotic cells. RM, receptor muscle. Scale bar 30 $\mu$m.

and induced necrosis of neurons [Figs. 3(b)–8(b)] and glial cells [Figs. 3(d)–8(d)] and apoptosis of glial cells [Figs. 3(c)–8(c)].

### 3.1 *NF-κB*

PDT-induced necrosis of neurons increased by 41% [$p < 0.05$; Fig. 3(b)] in the presence of NF-κB activator BA, whereas

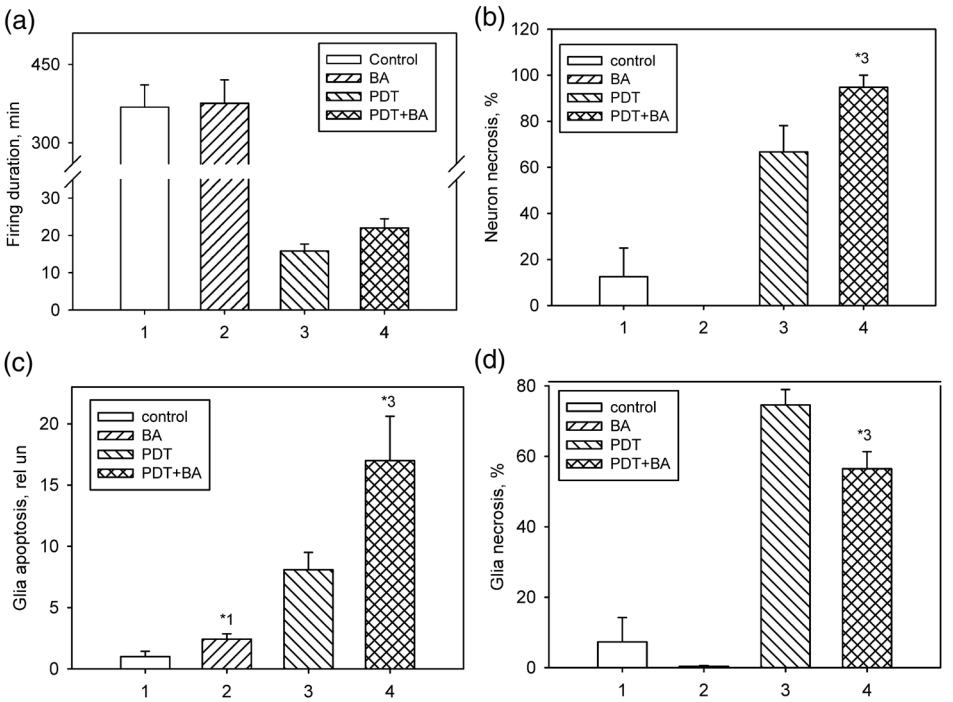


**Fig. 3** Effects of the activator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), betulinic acid (BA, 5 $\mu$M), on (a) firing duration; necrosis of (b) neurons, (c) glial apoptosis, and (d) glial cells. Significant difference from the indicated bar: $*p < 0.05$.

inhibition of NF-κB by CAPE or parthenolide significantly reduced neuronal necrosis by 43% and 33%, respectively [$p < 0.05$; Figs. 4(b) and 5(b)]. This indicated involvement of NF-κB in PDT-induced necrosis of MRN. By contrast, in glial cells, PDT-induced necrosis was reduced by 35% ($p < 0.05$) under NF-κB activation by BA, but it increased by 43% ($p < 0.01$) when NF-κB was inhibited by parthenolide [Figs. 3(d) and 5(d)]. CAPE was ineffective. Therefore, unlike neurons, NF-κB mediated protection of glial cells from PDT-induced necrosis. The level of PDT-induced apoptosis of glial

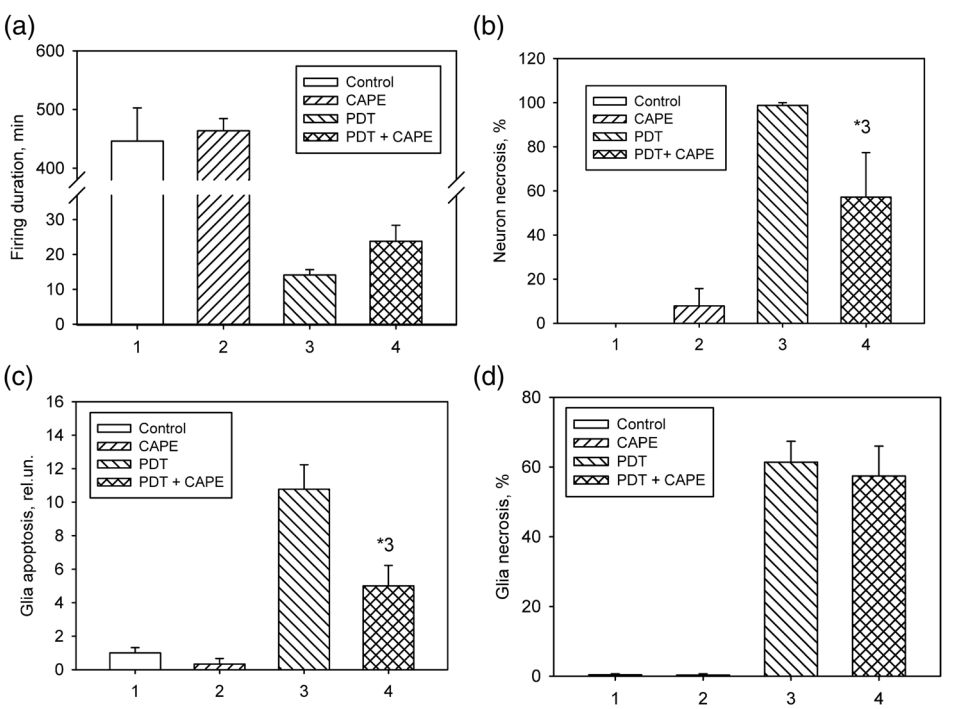


**Fig. 4** Effects of the inhibitor of NF-κB, caffeic acid phenethyl ester (CAPE, 30 $\mu$M), on (a) firing duration, necrosis of (b) neurons, (c) glial apoptosis, and (d) glial cells. Significant difference from the indicated bar: $*p < 0.05$.

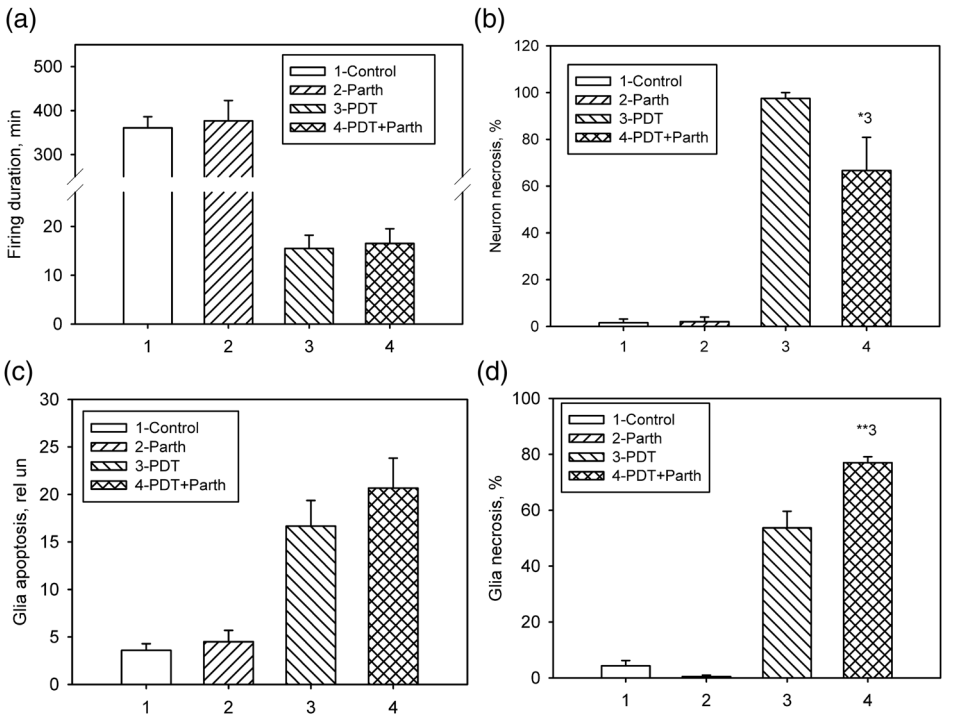


**Fig. 5** Effects of the inhibitor of NF-κB, parthenolide (Parth, 20 $\mu$M), on (a) firing duration, necrosis of (b) neurons, (c) glial apoptosis and (d) glial cells. Significant difference from the indicated bar: $*p < 0.05$; $**$ $p < 0.01$.

cells increased twofold ($p < 0.05$) when NF-κB was activated by BA [Fig. 3(c)], but decreased 2.1 fold in the presence of NF-κB inhibitor CAPE [Fig. 4(c)]. Another NF-κB inhibitor, parthenolide, was ineffective [Fig. 5(c)]. All of this indicated the involvement of NF-κB in PDT-induced apoptosis of glial cells.

### 3.2 *Activator Protein-1*

Inhibition of AP-1 by SR11302 (10 $\mu$M) did not significantly influence the PDT-induced shortening of neuronal activity and necrosis of neurons and glial cells [Figs. 6(a), 6(b), and 6(d)]. However, the level of PDT-induced apoptosis of glial cells decreased by 64% in the presence of SR11302 [$p < 0.05$; Fig. 6(c)] indicating the involvement of AP-1 in PDT-induced apoptosis of glial cells.

### 3.3 *STAT-3*

The levels of PDT-induced necrosis of neurons and glial cells decreased 4.6 and 1.8 fold, respectively, under inhibition of STAT-3 by 1 $\mu$M stattic [$p < 0.001$ and $p < 0.01$; Figs. 7(b) and 7(d)]. Likewise, PDT-induced apoptosis of glial cells was reduced 3.3 fold in the presence of stattic [$p < 0.05$; Fig. 7(c)]. Another inhibitor of STAT-3 cucurbitacin (50 nM) was less efficient. It did not influence PDT-induced necrosis of neurons and glial cells [Figs. 8(b) and 8(d)]. However, it prevented PDT-induced apoptosis of glial cells [Fig. 8(c)]. It is of interest that stattic shortened the duration of neuronal activity by 60% [$p < 0.05$; Fig. 7(a)].

## 4 Discussion

The present data summarized in Table 2 showed that modulators of all studied transcription factors: NF-κB, AP-1, and STAT-3 differently influenced PDT-induced death of neurons and glial cells.

These data indicate the involvement of NF-κB in PDT-induced necrosis of crayfish MRNs and apoptosis of satellite glial cells. At the same time, NF-κB showed the antinecrotic activity in glial cells. Therefore, signaling pathways that control cell death in crayfish neurons and glial cells differed.

NF-κB is ubiquitously expressed in all cell types in the nervous system: neurons, astrocytes, oligodendrocytes, Schwann cells, and microglia. It acts as a central integrator of stress responses and cell survival pathways. Its dual roles in survival and death of neurons and glial cells, protective or destructive, depend on types, developmental stages of cells, and pathological conditions. Being activated by diverse stimuli (more than 150), NF-κB regulates expression of a broad array of target genes (over 150) involved in cell death or survival.[35–39] The list of pro-survival proteins upregulated by NF-κB includes antiapoptotic protein Bcl-2, Mn-superoxide dismutase, inhibitor of apoptosis (IAP), survivin, $Ca^{2+}$/calmodulin-dependent protein kinase II (CamKII), etc. At the same time, NF-κB promotes expression of proapoptotic proteins such as p53, Bcl-Xs, Bax, c-Myc, inducible nitric oxide synthase (iNOS), etc.[38,39]

There are a number of reports in the literature on the involvement of NF-κB in apoptosis of glial cells under stress conditions. Vollgraf et al.[40] have reported the involvement of NF-κB in apoptosis of cultured rat brain oligodendrocytes induced by $H_2O_2$-mediated oxidative stress.[40] Ischemic/reperfusion injury of the mouse brain has been shown to be associated with NF-κB activation, following overexpression of p53, proapoptotic proteins—Bax and caspase-3, and down-regulation of antiapoptotic protein Bcl-2.[41] NF-κB and $Ca^{2+}$-dependent enzymes, calpain and calcineurin, were involved in reperfusion-induced apoptosis of astrocytes.[42]

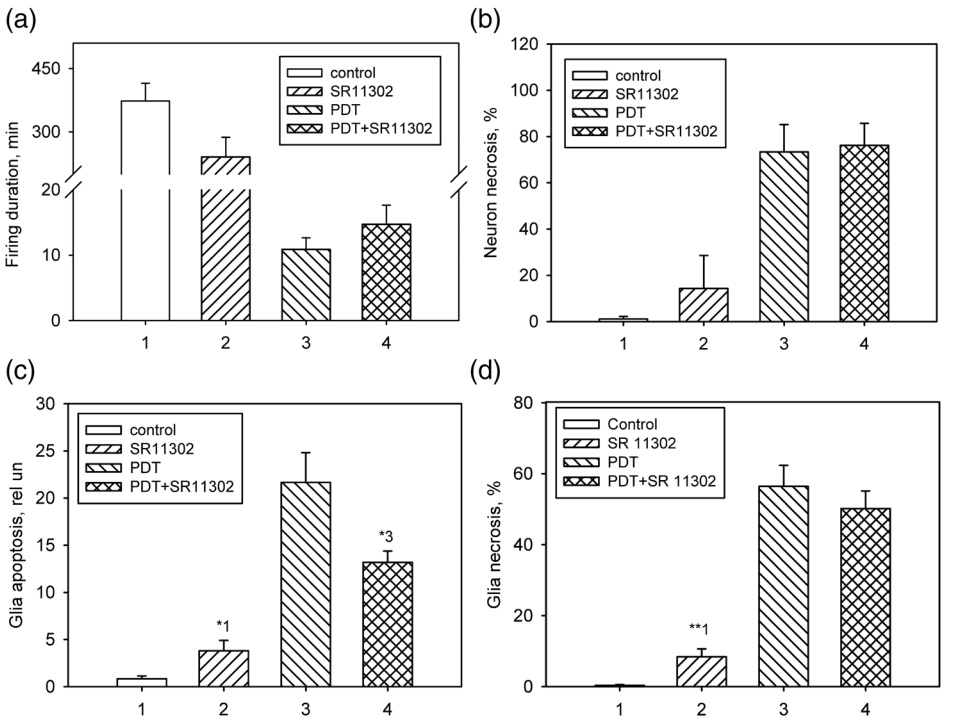


**Fig. 6** Effects of activator protein-1 inhibitor SR11302 (10 $\mu$M), on (a) firing duration, necrosis of (b) neurons, (c) glial apoptosis and (d) glial cells. Significant difference from the indicated bar: $*p < 0.05$; $**$ $p < 0.01$.

PDT has been reported to activate NF-κB in various cell lines[19–22] and facilitate its binding to DNA.[22] PDT-induced activation of NF-κB caused apoptosis of cultured cancer cells.[21] The present proapoptotic effect of NF-κB on photosensitized crayfish glial cells is in line with these findings. This effect could be mediated by iNOS-produced NO and protein kinase G, which also mediated PDT-induced apoptosis of crayfish glial cells:[33] PDT → NF-κB → iNOS → NO → protein kinase G → apoptosis. It should be mentioned that in glioblastoma cells, PDT inhibited or down-regulated NF-κB.[43,44]

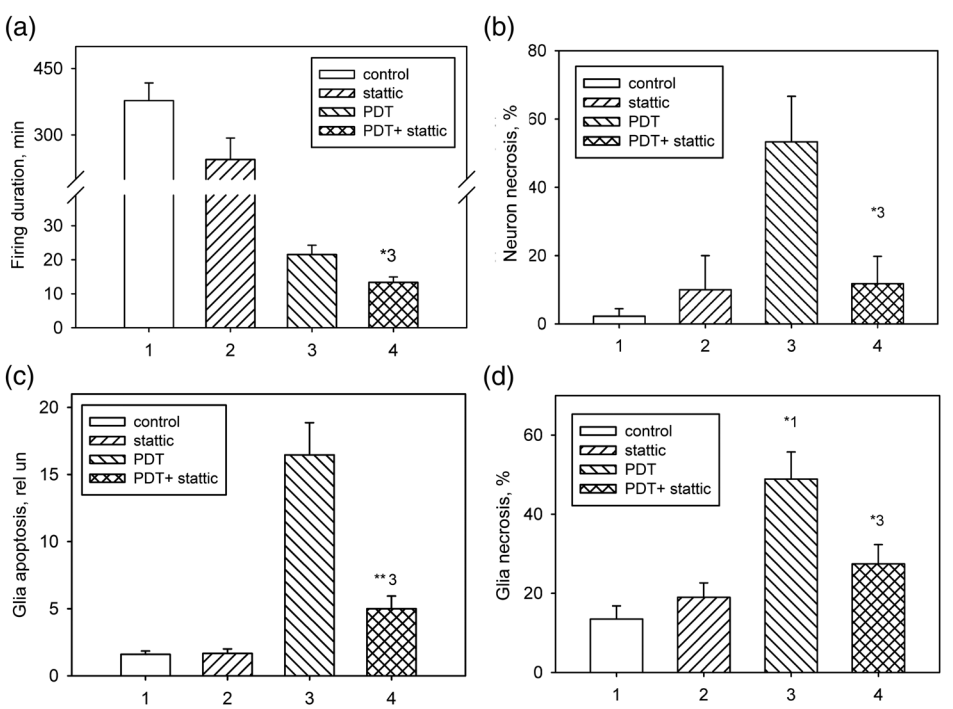


**Fig. 7** Effects of STAT-3 inhibitor stattic (1 $\mu$M) on (a) firing duration, necrosis of (b) neurons, (c) glial apoptosis, and (d) glial cells. Significant difference from the indicated bar: $*p < 0.05$; $**$ $p < 0.01$.

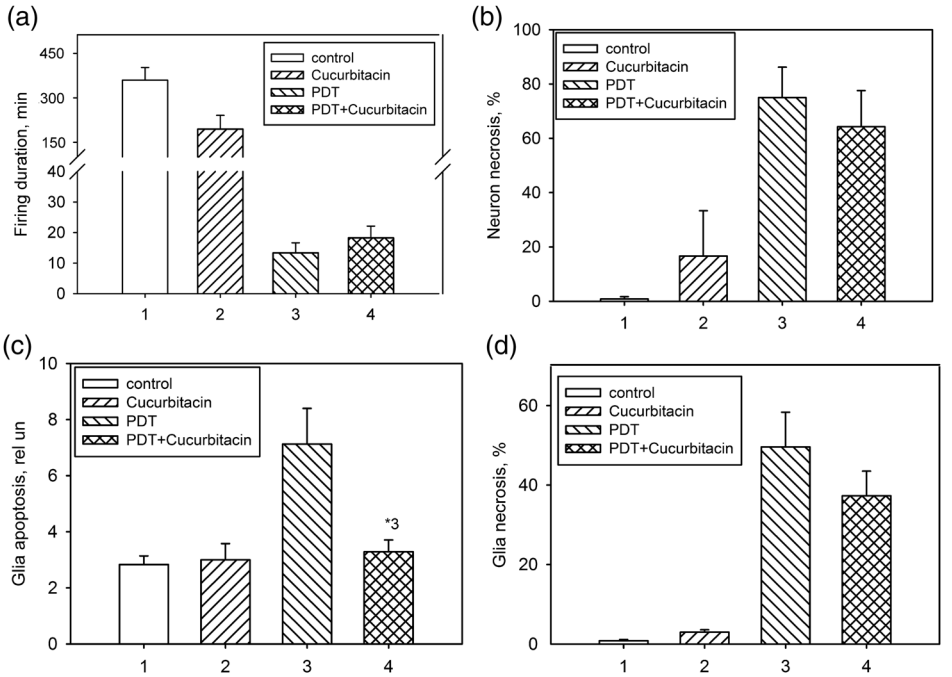


**Fig. 8** Effects of STAT-3 inhibitor cucurbitacin (50 nM), on (a) firing duration, necrosis of (b) neurons, (c) glial apoptosis and (d) glial cells. Significant difference from the indicated bar: $*p < 0.05$.

The role of NF-κB in necrosis of neurons and glial cells is less studied compared to apoptosis. As shown earlier, exogenous neurotrophic factors, NGF and GDNF, protected crayfish glial cells against PDT-induced necrosis.[45,46] Similarly, the pathway GDNF/ERK/NF-kB has been shown to mediate the protection of brain astrocytes against ischemia-induced death.[47] The observed pronecrotic activity of NF-kB in photosensitized crayfish neurons could be mediated by signaling proteins, such as adenylate cyclase,[30] calmodulin and CamKII,[30] or protein kinase B/Akt,[32] which were similarly involved in PDT-induced necrosis of this neuron. As recently reported, the dysfunction of Hsp70.1 leads to activation of NF-κB signaling and necrosis of neurons.[48]

**Table 2** The summary data on the effects of modulators of transcription factors—nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κb), activator protein-1 (AP-1), and signal transducer and activator of transcription-3 (STAT-3)—on PDT-induced abolition of neuronal activity, necrosis of neurons and glial cells and glial apoptosis. ↓, significant decrease ($p < 0.05$); ↑, significant increase ($p < 0.05$); –, absence of effects ($p > 0.05$).

| Modulator | Modulator concentration | Firing duration | Neuron necrosis | Glia necrosis | Glia apoptosis |
|---|---|---|---|---|---|
| | | NF-κB | | | |
| Betulinic acid (activator) | 5 μM | – | ↑ | ↓ | ↑ |
| CAPE (inhibitor) | 30 μM | – | ↓ | – | ↓ |
| Parthenolide (inhibitor) | 20 μM | – | ↓ | ↑ | – |
| | | AP-1 | | | |
| SR11302 (Inhibitor) | 10 μM | – | – | – | ↓ |
| | | STAT-3 | | | |
| Stattic (Inhibitor) | 1 μM | ↓ | ↓ | ↓ | ↓ |
| Cucurbitacine (inhibitor) | 50 nM | – | – | – | ↓ |

Note: CAPE, caffeic acid phenethyl ester.

Transcription factor AP-1, like NF-κB, is activated at the early stages of the cell response to stress. Its inhibition by SR11302 in our experiments reduced PDT-induced apoptosis of glial cells but did not influence necrosis of neurons and glia. This indicated involvement of AP-1 in PDT-induced glial apoptosis but not in necrotic processes in neurons and glia. This is in agreement with the proapoptotic role of AP-1 in rat oligodendrocytes subjected to hydrogen peroxide-mediated oxidative stress.[40] Photodynamic treatment caused strong and prolonged activation of AP-1 in the epithelial cell line HeLa[24,25] and squamous cell carcinoma cell line A-431.[49]

The present work indicated involvement of transcription factor STAT-3 in apoptosis of crayfish glial cells and in necrosis of MRN and satellite glia. There are very little data on the role of the signaling pathway Jak/STAT in photodynamic cell injury in the literature. PDT has been shown to induce crosslinking of STAT-3 protein in epithelial cells.[50] Solár et al.[51] showed that erythropoietin-stimulated Jak2/STAT pathway increased the resistance of ovarian cancer cells to photodynamic injury. These results are not in agreement with our data. The molecular mechanism of STAT-3 involvement in PDT-induced death of neurons and glial cells remains to be studied in the future.

Thus, transcription factors—NF-κB, AP-1, and STAT-3—influenced PDT-induced death and survival of crayfish MRNs and satellite glial cells. Nuclear transcription factor, NF-κB, was involved in PDT-induced necrosis of neurons and apoptosis of glial cells. At the same time, NF-κB played the antinecrotic role in glial cells. AP-1 did not influence PDT-induced necrosis of neurons and glia but mediated glial apoptosis. Signal

transducer and activator of transcription STAT-3 participated in PDT-induced necrosis of neurons and glial cells as well as in apoptosis of glial cells. The signaling pathways that regulate cell death and survival in crayfish neurons and glial cells are different. Using different inhibitors or activators of the studied transcription factors, one can differently influence the sensitivity and resistance of neurons and glial cells to PDT.

## Acknowledgments

The study was supported by the Russian Foundation of Basic Research (Grant Nos. 11-04-01476, 14-04-32270, and 14-04-00741) and Russian Ministry of Education and Science (Research organization #790). The authors declare no conflicts of interest.

## References

1. A. A. Krasnovsky, "Singlet oxygen and primary mechanisms of photodynamic therapy and photodynamic diseases," in *Photodynamic Therapy at the Cellular Level*, A. B. Uzdensky, Ed., pp. 17–62, Research Signpost, Trivandrum (2007).
2. A. P. Castano, T. N. Demidova, and M. R. Hamblin, "Mechanisms in photodynamic therapy: part two—cellular signaling, cell metabolism and modes of cell death," *Photodiagn. Photodyn. Ther.* **2**, 1–23 (2005).
3. A. B. Uzdensky, "Signal transduction and photodynamic therapy," *Curr. Signal Transduction Ther.* **3**, 55–74 (2008).
4. S. Madsen and H. Hirschberg, "Photodynamic therapy and detection of high-grade gliomas," *J. Environ. Pathol. Toxicol. Oncol.* **25**, 453–466 (2006).
5. S. S. Stylli and A. H. Kaye, "Photodynamic therapy of cerebral glioma—a review. Part II: clinical studies," *J. Clin. Neurosci.* **13**, 709–717 (2006).
6. S. Eljamel, "Photodynamic applications in brain tumors: a comprehensive review of the literature," *Photodiagn. Photodyn. Ther.* **7**, 76–85 (2010).
7. H. Kostron, "Photodynamic diagnosis and therapy and the brain," *Methods Mol. Biol.* **635**, 261–280 (2010).
8. D. Bechet et al., "Photodynamic therapy of malignant brain tumours: a complementary approach to conventional therapies," *Cancer Treat. Rev.* **40**, 229–241 (2014).
9. M. C. Tetard et al., "Experimental use of photodynamic therapy in high grade gliomas: a review focused on 5-aminolevulinic acid," *Photodiagn. Photodyn. Ther.* **11**, 319–330 (2014).
10. R. D. Almeida et al., "Intracellular signaling mechanisms in photodynamic therapy," *Biochim. Biophys. Acta* **1704**, 59–86 (2004).
11. E. Buytaert, M. Dewaele, and P. Agostinis, "Molecular effectors of multiple cell death pathways initiated by photodynamic therapy," *Biochim. Biophys. Acta* **1776**, 86–107 (2007).
12. M. Dewaele et al., "Death and survival signals in photodynamic therapy," *Methods Mol. Biol.* **635**, 7–33 (2010).
13. N. Li and M. Karin, "Is NF-κB the sensor of oxidative stress," *FASEB J.* **13**, 1137–1143 (1999).
14. A. Oeckinghaus and S. Ghosh, "The NF-κB family of transcription factors and its regulation," *Cold Spring Harb. Perspect. Biol.* **1**(4), a000034 (2009).
15. M. J. Morgan and Z. G. Liu, "Crosstalk of reactive oxygen species and NF-κB signaling," *Cell Res.* **21**(1), 103–115 (2011).
16. A. Siomek, "NF-κB signaling pathway and free radical impact," *Acta Biochim. Pol.* **59**(3), 323–331 (2012).
17. M. Ameyar, M. Wisniewska, and J. B. Weitzman, "A role for AP-1 in apoptosis: the case for and against," *Biochimie* **85**(8), 747–752 (2003).
18. J. S. Rawlings, K. M. Rosler, and D. A. Harrison, "The JAK/STAT signaling pathway," *J. Cell Sci.* **117**, 1281–1283 (2004).
19. C. Volanti, J. Y. Matroule, and J. Piette, "Involvement of oxidative stress in NF-kappaB activation in endothelial cells treated by photodynamic therapy," *Photochem. Photobiol.* **75**(1), 36–45 (2002).
20. J. Y. Matroule, C. Volanti, and J. Piette, "NF-κB in photodynamic therapy: discrepancies of a master regulator," *Photochem. Photobiol.* **82**(5), 1241–1246 (2006).
21. J. Y. Matroule et al., "Role of nuclear factor-kappa B in colon cancer cell apoptosis mediated by aminopyropheophorbide photosensitization," *Photochem. Photobiol.* **70**, 540–548 (1999).
22. S. W. Ryter and C. J. Gomer, "Nuclear factor kappa B binding activity in mouse L1210 cells following photofrin II-mediated photosensitization," *Photochem. Photobiol.* **58**(5), 753–756 (1993).
23. H. M. Chen et al., "5-aminolevulinic acid induce apoptosis via NF-κB/JNK pathway in human oral cancer Ca9-22 cells," *J. Oral. Pathol. Med.* **40**(6), 483–489 (2011).
24. G. Kick et al., "Strong and prolonged induction of c-jun and c-fos proto-oncogenes by photodynamic therapy," *Br. J. Cancer.* **74**(1), 30–36 (1996).
25. G. Kick et al., "Photodynamic therapy induces expression of interleukin 6 by activation of AP-1 but not NF-kappa B DNA binding," *Cancer Res.* **55**(11), 2373–2379 (1995).
26. J. S. Rawlings, K. M. Rosler, and D. A. Harrison, "The JAK/STAT signaling pathway," *J. Cell Sci.* **117**, 1281–1283 (2004).
27. G. M. Fedorenko and A. B. Uzdensky, "Ultrastructure of neuroglial contacts in crayfish stretch receptor," *Cell Tissue Res.* **337**, 477–490 (2009).
28. A. Uzdensky et al., "Photodynamic inactivation of isolated crayfish mechanoreceptor neuron," *Photochem. Photobiol.* **76**, 431–437 (2002).
29. A. Uzdensky et al., "Involvement of adenylate cyclase and tyrosine kinase signaling pathways in response of crayfish stretch receptor neuron and satellite glia cell to photodynamic treatment," *Glia* **49**, 339–348 (2005).
30. A. Uzdensky et al., "Involvement of $Ca^{2+}$- and cyclic adenosine monophosphate-mediated signaling pathways in photodynamic injury of isolated crayfish neuron and satellite glial cells," *J. Neurosci. Res.* **85**, 860–870 (2007).
31. A. V. Lobanov and A. B. Uzdensky, "Protection of crayfish glial cells but not neurons from photodynamic injury by nerve growth factor," *J. Mol. Neurosci.* **39**, 308–319 (2009).
32. M. A. Komandirov et al., "On the role of phosphatidylinositol 3-kinase, protein kinase B/Akt, and glycogen synthase kinase-3$\beta$ in photodynamic injury of crayfish neurons and glial cells," *J. Mol. Neurosci.* **45**, 229–235 (2011).
33. V. D. Kovaleva et al., "Involvement of nitric oxide in photodynamic injury of neurons and glial cells," *Nitric oxide* **29**, 46–52 (2013).
34. S. C. Benn and C. J. Woolf, "Adult neuron survival strategies-slamming on the brakes," *Nat. Rev. Neurosci.* **5**, 686–700 (2004).
35. M. P. Mattson, "NF-kappaB in the survival and plasticity of neurons," *Neurochem. Res.* **30**, 883–893 (2005).
36. S. Camandola and M.P. Mattson, "NF-kappa B as a therapeutic target in neurodegenerative diseases," *Expert. Opin. Ther. Targets* **11**(2), 123–132 (2007).
37. B. Kaltschmidt, D. Widera, and C. Kaltschmidt, "Signaling via NF-kappaB in the nervous system," *Biochim. Biophys. Acta* **1745**(3), 287–299 (2005).
38. Z. Qin, L. Tao, and X. Chen, "Dual roles of NF-κB in cell survival and implications of NF-κB inhibitors in neuroprotective therapy," *Acta Pharmacol. Sin.* **28**, 1859–1872 (2007).
39. H. L. Pahl, "Activators and target genes of Rel/NF-kappaB transcription factors," *Oncogene* **18**, 6853–6866 (1999).
40. U. Vollgraf, M. Wegner, and C. Richter-Landsberg, "Activation of AP-1 and nuclear factor-kappaB transcription factors is involved in hydrogen peroxide-induced apoptotic cell death of oligodendrocytes," *J. Neurochem.* **73**(6), 2501–2509 (1999).
41. J. H. Gu et al., "Inhibition of NF-κB activation is associated with anti-inflammatory and anti-apoptotic effects of Ginkgolide B in a mouse model of cerebral ischemia/reperfusion injury," *Eur. J. Pharm. Sci.* **47**, 652–660 (2012).
42. K. Takuma, A. Baba, and T. Matsuda, "Astrocyte apoptosis: implications for neuroprotection," *Prog. Neurobiol.* **72**(2), 111–127 (2004).
43. S. Karmakar et al., "5-Aminolevulinic acid-based photodynamic therapy suppressed survival factors and activated proteases for apoptosis in human glioblastoma U87MG cells," *Neurosci. Lett.* **415**(3), 242–247 (2007).
44. I. Coupienne et al., "NF-kappaB inhibition improves the sensitivity of human glioblastoma cells to 5-aminolevulinic acid-based photodynamic therapy," *Biochem. Pharmacol.* **81**(5), 606–616 (2011).
45. A. V. Lobanov and A. B. Uzdensky, "Protection of crayfish glial cells but not neurons from photodynamic injury by nerve growth factor," *J. Mol. Neurosci.* **39**, 308–319 (2009).

46. A. Uzdensky et al., "Protection effect of GDNF and neurturin on photosensitized crayfish neurons and glial cells," *J. Mol. Neurosci.* **49**, 480–490 (2013).
47. L. F. Chu et al., "Ischemic brain cell-derived conditioned medium protects astrocytes against ischemia through GDNF/ERK/NF-kB signaling pathway," *Brain Res.* **1239**, 24–35 (2008).
48. T. Yamashima, "Hsp70.1 and related lysosomal factors for necrotic neuronal death," *J. Neurochem.* **120**(4), 477–494 (2012).
49. T. Verwanger et al., "Gene expression pattern following photodynamic treatment of the carcinoma cell line A-431 analysed by cDNA arrays," *Int. J. Oncol.* **21**(6), 1353–1359 (2002).
50. W. Liu, A. R. Oseroff, and H. Baumann, "Photodynamic therapy causes cross-linking of signal transducer and activator of transcription proteins and attenuation of interleukin-6 cytokine responsiveness in epithelial cells," *Cancer Res.* **64**(18), 6579–6587 (2004).
51. P. Solár et al., "Erythropoietin inhibits apoptosis induced by photodynamic therapy in ovarian cancer cells," *Mol. Cancer Ther.* **7**, 2263–2271 (2008).

**Elena Berezhnaya** is a PhD student at the Southern Federal University (Rostov-on-Don, Russia).

**Marya Neginskaya** is a PhD student at the Southern Federal University (Rostov-on-Don, Russia).

**Vera Kovaleva** is a PhD student at the Southern Federal University (Rostov-on-Don, Russia).

**Svetlana Sharifulina** is a PhD student at the Southern Federal University (Rostov-on-Don, Russia).

**Irina Ischenko** is a PhD student at the Southern Federal University (Rostov-on-Don, Russia).

**Maxim Komandirov** is a PhD student at the Southern Federal University (Rostov-on-Don, Russia).

**Mikhail Rudkovslii** is an associate professor in biophysics at the Southern Federal University (Rostov-on-Don, Russia). He received his PhD in physiology in 2004. He is the author of 12 journal papers. His current research interests include photobiology and neuroscience.

**Anatoly B. Uzdensky** is a professor in biophysics and head of the Laboratory of Molecular Neurobiology at the Southern Federal University (Rostov-on-Don, Russia). He received his PhD and DSc degrees in biophysics in 1980 and 2005, respectively. He is the author of more than 80 journal papers and 2 books. His current research interests include photobiology, neuroscience, cell biology, and proteomics. He is a member of SPIE, European, Russian, and American Societies for Phiotobiology.