

Supplementary Figure 1.

Axonal/dendritic degeneration occurs well before a significant increase in cell body death occurs in SA treated versus controls

We conducted a cell viability assay (LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit , Molecular Probes, Eugene, OR) on DIV16 DIV19 and DIV22 of cultures treated with SA. The results showed that the number of dead cells (stained red) on day 16 was less than 35% (A). Loss of the projections at this stage was significant (B). By DIV19 the number of dead cells increased to 60% (C), whereas virtually all the projections were fragmented at this stage (D). On DIV22 the culture had no visible network (F), and 95% of the cells were dead. Control cultures on DIV22 still had well established network of the projections (H) and number of dead cells was less than 50%. The cell death scores obtained from three independent experiments are included in the main text at page 12.

Supplementary Figure 2.

Increased H₂O₂-induced ROS production in heme-deficient neurons.

Change of 2',7'-dichlorofluorescein (DCF), the fluorescent product of 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF-DA) (Molecular Probes Inc. Eugene, OR, USA), was used to monitor the H₂O₂-induced generation of ROS by BD FACSCalibur

flow cytometer ((BD-Biosciences), excitation at 488 nm and emission at 519 nm, as described previously (Boldyrev et al., 2004; Lee et al., 2005). The uptake of H₂DCF-DA (final concentration 20 µM) dissolved in DMSO (final DMSO concentrations in the samples were not higher than 0.01%) was carried out for 45 minutes and followed by the incubation with 50 µM H₂O₂ in growing medium for 20 minutes. Samples contained 200 000–300 000 cells and 10 000 events in each sample were assayed. The data were analysed using Cell Quest TM software (Becton-Dickinson, San Jose, CA). Viability of the cells, tested with propidium iodide fluorescence was approximately 90% of the total cell populations in control and SA-treated cultures.

(A) Representative histogram of three independent experiments reflecting the overlapping sub-populations of viable neurons in control (thin line) and SA treated (thick line) cultures, M1 gates highly fluorescent neurons. (B) The percent of M1 cells in SA treated culture was increased to 51.79±3.51 compared to 31.37±3.01 in control neurons (p<0.05).

References.

- Boldyrev A, Bulygina E, Leinsoo T, Petrushanko I, Tsubone S, Abe H (2004) Protection of neuronal cells against reactive oxygen species by carnosine and related compounds. *Comp Biochem Physiol B Biochem Mol Biol* 137:81-88.
- Lee HJ, Ban JY, Seong YH (2005) Blockade of 5-HT(3) receptor with MDL7222 and Y25130 reduces hydrogen peroxide-induced neurotoxicity in cultured rat cortical cells. *Life Sci* 78:294-300.

