The Maestro microelectrode array (MEA, Axion BioSystems) system was used to measure spontaneous activity of dopaminergic neurons. 96-well MEA plates were precoated with 0.1 mg/ml poly-D-lysine hydrobromide (Sigma-Aldrich) in 0.1 M borate buffer (Sigma) for 1 h at RT. 1\*10^5 hNESCs were placed onto each array in a Matrigel (Corning) droplet and incubated for 1 h at 37 °C before adding culturing media. Cells were recorded during the course of dopaminergic differentiation at a sampling rate of 12.5 kHz for 10 min at 37 °C. Axion Integrated Studio (AxIS 2.1) was used to process the raw data as previously described (Monzel et al., 2017).

* *Using Axion Integrated Studio (AxIS 2.1), a Butterworth band pass filter with 200-3000 Hz cutoff frequency and a threshold of 6 x SD were set to minimize both false-positives and missed detections. The Neural Metric Tool (Axion BioSystems) was used to analyze the spike raster plots. Electrodes with an average of ≥5 spikes/min were defined as active. The spike count files generated from the recordings were used to calculate the number of spikes/active electrode/measurement. Further details regarding the MEA system were previously described (Bardy et al., 2015).*

For the pharmacological treatment (n=3) neurons were consecutively treated with Gabazine, D-AP-5, NBQX (Cayman Chemical, end concentration: 50 mM each), and Quinpirole (Sigma Aldrich, end concentration: 5µM) after 48 days of differentiation. Tetrodotoxin (TTX, Cayman Chemical, 1 µM) was applied at the end for blocking and hence verifying the neuronal activity. The spike count files generated from the recordings were used to calculate the number of spikes/active electrode. Further details regarding the MEA system were previously described by Bardy et al. (2015).