A novel human iPSC-based co-culture model to study neurocardiac interaction *in vitro*

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The cardiac autonomic nervous system is involved in many cardiac disorders. However, the neuronal regulation of the heart in these diseases remains poorly understood mainly due to the lack of proper human cell models. To overcome this limitation, we have created an *in vitro* neurocardiac model uniquely based on human Induced Pluripotent Stem Cell (iPSC)-derived cells, namely iPSC-Cardiomyocytes (iPSC-CMs) and iPSC-Sympathetic Neurons (iPSC-SNs). iPSC-SNs in monoculture were characterized for MAP-2 (neuronal marker), for TH and DBH (adrenergic lineage markers), and for peripherin (peripheral nervous system marker) by immunofluorescence and western blot analyses. Quantification of TH+/DBH+ double positive cells at day 30 using flow cytometry showed 71-90% of positivity. iPSC-SNs exhibited spontaneous firing and burst activity measured using the Maestro Edge Multi-Electrode Array (MEA). iPSC-CMs and iPSC-SNs were co-cultured in two chambers of a silicon insert and, after insert removal, iPSC-SNs formed axons projecting towards the CMs. The beat amplitude of iPSC-CMs was measured using the MEA system and was significantly increased after 7 days of co-culture (monoculture 0.65%±0.04 *vs* co-culture 2.20% ± 0.14; $p<0.0001$), although the beat rate was stable. Of note, a significant increase in the beat rate of iPSC-CMs in co-culture was observed after nicotine treatment (baseline 53 BPM±8 *vs* nicotine 79 BPM±12; $p=0.0034$), that had no effect on iPSC-CMs in monoculture. On the contrary, after treatment with α-bungarotoxin, a toxin binding to nicotinic receptors and blocking neural transmission, the beat rate of iPSC-CMs in co-culture was unaffected thus confirming the capability of iPSC-SNs to establish functional connections with iPSC-CMs. The proposed neurocardiac system provides a promising modelling tool for a wide range of cardiac pathologies, as well as for drug screening and personalized medicine approach.

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