



Temporal changes in key developmental transcription factors in dopamine neurons during MPP⁺ induced injury and recovery in zebrafish brain

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Supplementary Tables

Supplementary Table 1 — Primer pairs used in PCR preparing for WISH probes

Gene Name	Sequence Details (5'-3')	Size
Foxa1	Forward primer-CAGGCACTCGTTGTCGTTCA	129bp
	Reverse primer-CAGGTAACAGCCATTCTCA	
Nurr1	Forward primer-TGAAGCCTCCCTGCCTCTAC	103bp
	Reverse primer-GATGGTGCTGCTGCTGATG	
En-1a	Forward primer-GCGACGGTGTTTCTTCTT	123bp
	Reverse primer-TCTGCTTCTGGCTGTCTT	
lmx1a	Forward primer -GCAAGCGAGACTACCAGAA	130bp
	Reverse primer-ACACAGCAGCAGAAGCAG	
TH(Probe)	5'/5DigN/GGCAAUGUCUCCGAUCAUCUCCGU-3'	--
DAT(Probe)	5'/5DigN/CCCAAACCCGACUCCAGAGAAA-3'	--

Supplementary Table S2 — Target genes, Primer pairs, amplicon length and annealing temperatures

S.No	Primer Name	Primer Details (5'-3')	Product Size (bp)	Annealing Temperature (°C)
1	Foxa1	Forward primer-CAGGCACTCGTTGTCGTTCA	129	53
2		Reverse primer-CAGGTAACAGCCATTCTCA		
3	Nurr1	Forward primer-GAAGCCTCCCTGCCTCTAC	103	53
4		Reverse primer-GATGGTGCTGCTGCTGATG		
5	En-1a	Forward primer-GCGACGGTGTTTCTTCTT	123	52
6		Reverse primer-TCTGCTTCTGGCTGTCTT		
7	En-2a	Forward primer-CTTCCACAACGCATACGA	103	52
8		Reverse primer-TCTGAACCCAAGCACTGA		
9	lmx1 A	Forward primer-GCAAGCGAGACTACCAGAA	130	59.6
10		Reverse primer-ACACAGCAGCAGAAGCAG		
11	lmx1 B	Forward primer-ACAGCACTTCTCGTCCTC	128	59.6
12		Reverse primer-GGCGTTCACCATCATTATCG		
13	En-2 B	Forward primer-AGAGAACCGCTGTCCATC	124	58.6
14		Reverse primer-CAGAGATTCGTCCTGCTA		
15	En-1 B	Forward primer-TTCTTCGTCCTCGTCGTCTT	121	58.6
16		Reverse primer-GCGTTCACCATCATTATCG		
17	TH	Forward primer-CTGTTTCAGCCATACCAAGAC	162	60
18		Reverse primer-GGTTGTCCAGCACTTCTATG		
19	DAT	Forward primer-GGTCAGTATAACCGTGAAGG	155	60
20		Reverse primer-AGAAGGAGGAGAACAGGTA		
21	B-Actin	Forward primer-GGACTCTGGTGTGGTGTGA	129	59.6
22		Reverse primer-GCTCGGTCAGGATCTTCAT		

Supplementary Table S3 — Trends of relative gene expression ($2^{-\Delta Ct}$) of 10 genes during embryonic exposure of 1mM MPP⁺. Quantitative Data represents as a Mean±SE (n=3). ^{a, b, c} Mean ± SE with different superscripts differ significantly ($P < 0.005$)

Gene	24 Hpf		48 Hpf		72 Hpf		96 Hpf	
	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺
Nurr1	0.004±0.0002	0.001±0.0002 ^a	0.008±0.00043	0.002±0.00012 ^{ab}	0.012±0.00034	0.0025±0.00013 ^{ab}	0.022±0.00042	0.003±0.00011 ^{ab}
Foxa1	0.0093±0.0001	0.022±0.0002 ^a	0.0094±0.0004	0.0022±0.00023 ^b	0.013±0.00051	0.002±0.00043 ^a	0.031±0.00053	0.004±0.00019 ^a
En-1a	0.002±0.00012	0.0012±0.001 ^a	0.004±0.00053	0.001±0.00013 ^b	0.005±0.00034	0.001±0.00054 ^b	0.006±0.0006	0.001±0.00023 ^b
En-1b	0.006±0.00021	0.002±0.0005 ^a	0.007±0.0002	0.002±0.00053 ^b	0.008±0.0003	0.002±0.00044 ^b	0.009±0.00053	0.0022±0.00034 ^b
En-2a	0.002±0.00011	0.001±0.0001 ^{ac}	0.0035±0.0004	0.001±0.00023 ^b	0.0044±0.00042	0.001±0.00040 ^b	0.0055±0.0004	0.001±0.00042 ^b
En-2b	0.002±0.00031	0.0018±0.000 ^{ab}	0.003±0.0004	0.001±0.00023 ^b	0.004±0.0004	0.001±0.0004 ^b	0.005±0.0003	0.001±0.00043 ^b
Lmx1a	0.003±0.00022	0.002±0.00022 ^{ab}	0.004±0.00014	0.001±0.00013 ^b	0.005±0.00013	0.001±0.00032 ^b	0.0061±0.00011	0.0011±0.00046 ^b
Lmx1b	0.003±0.00012	0.002±0.00023 ^a	0.003±0.00032	0.001±0.00023 ^b	0.004±0.00021	0.001±0.00023 ^b	0.005±0.00021	0.001±0.00021 ^b
TH	0.0042±0.00012	0.001±0.0004 ^{ab}	0.0053±0.00012	0.001±0.00021 ^b	0.0061±0.00012	0.0011±0.00023 ^b	0.0088±0.00012	0.0013±0.00025 ^{ab}
DAT	0.00411±0.00012	0.00109±0.0003 ^{ab}	0.0078±0.00012	0.00145±0.00042 ^b	0.0119±0.00012	0.00154±0.0004 ^{ab}	0.0126±0.00012	0.00151±0.0002 ^{ab}

Supplementary Table S4 — Regulation of gene expression at different time points by MPP⁺ exposure (24 Hpf-96 Hpf) ($P < 0.05$)

Gene	24 Hpf	48 Hpf	72 Hpf	96 Hpf
Nurr1	-4.0	-4.0	-4.8	-7.3
Foxa1	-4.2	-4.3	-6.5	-7.8
En-1a	-1.5	-4.0	-5.0	-5.5
En-1b	-1.5	-3.0	-4.0	-5.0
En-2a	-2.0	-4.0	-5.0	-6.0
En-2b	-2.0	-3.5	-4.0	-4.1
Lmx1a	-2.0	-3.5	-4.4	-5.5
Lmx1b	-1.0	-3.0	-4.0	-5.0
TH	-4.2	-5.3	-5.5	-6.8
DAT	-3.8	-5.4	-7.7	-8.3

Supplementary Table S5 — Trends of relative gene expression ($2^{-\Delta Ct}$) of 10 genes in post injury. Quantitative Data represents as a Mean ± SE (n=3). ^{a, b, c} Mean ± SE with different superscripts differ significantly ($P < 0.005$)

Gene Name	Day1		Day2		Day3		Day4		Day5		Day6		Day7		Day8		Day8	
	Control	1 mM MPP ⁺	Control	1mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺	Control	1 mM MPP ⁺
Nurr1	0.025±0.00432	0.0034±0.00028 ^a	0.03±0.0045	0.004±0.00031 ^{ab}	0.04±0.0043	0.064±0.00041 ^{ab}	0.06±0.0043	0.01±0.00072 ^c	0.075±0.00032	0.014±0.00011 ^c	0.075±0.0005	0.021±0.00023 ^c	0.079±0.0004	0.029±0.00028 ^c	Male 0.031±0.0003	Male 0.029±0.00043	Female 0.028±0.0034	Female 0.031±0.0001
Foxa1	0.025±0.00063	0.0034±0.00029 ^a	0.03±0.0021	0.004±0.00041 ^{ab}	0.04±0.00062	0.064±0.00022 ^{ab}	0.06±0.00056	0.01±0.00053 ^{ab}	0.075±0.00053	0.014±0.00021 ^c	0.075±0.00043	0.021±0.00042	0.079±0.00045	0.029±0.00025 ^c	0.031±0.00023 ^c	0.029±0.00023	0.028±0.0022	0.031±0.00021
En-1a	0.0075±0.00064	0.001±0.00038 ^b	0.008±0.0003	0.001±0.00045 ^b	0.0085±0.00075	0.002±0.00021	0.009±0.00078	0.0032±0.00045 ^{ab}	0.01±0.00053	0.004±0.00034 ^{ab}	0.013±0.0004	0.005±0.00044 ^a	0.014±0.0004	0.006±0.00029 ^a	0.018±0.00042	0.016±0.00011	0.019±0.0002	0.015±0.00021
En-1b	0.011±0.00056	0.0023±0.00034 ^b	0.0015±0.00067	0.003±0.00034 ^b	0.003±0.00023	0.004±0.00053 ^b	0.004±0.00075	0.006±0.00031 ^a	0.007±0.00023 ^{ab}	0.007±0.0004	0.007±0.0004	0.007±0.0003 ^b	0.009±0.0003	0.009±0.00029 ^{ab}	0.036±0.0005	0.036±0.0002	0.038±0.0042	0.034±0.00022
En-2a	0.006±0.00034	0.001±0.00034 ^b	0.0062±0.00032	0.0013±0.00063 ^b	0.007±0.0002	0.0015±0.00053 ^b	0.0075±0.0001	0.0019±0.00034 ^b	0.0078±0.0003	0.0021±0.00034 ^b	0.008±0.0006	0.0026±0.0004 ^b	0.008±0.00049 ^b	0.0028±0.00024	0.02±0.00022	0.016±0.00043	0.019±0.0003	0.018±0.0003
En-2b	0.0053±0.0002	0.001±0.00042 ^b	0.0058±0.00032	0.001±0.00045 ^b	0.006±0.00011	0.0018±0.00064 ^b	0.006±0.0001	0.0018±0.00021 ^b	0.0066±0.00023	0.0022±0.00030 ^b	0.0068±0.0001	0.0022±0.0002 ^a	0.007±0.00021	0.0025±0.00048 ^c	0.007±0.0004	0.007±0.00011	0.027±0.00023	0.023±0.0004
Lmx1a	0.0072±0.00033	0.0011±0.00045 ^b	0.0075±0.00054	0.0012±0.00054 ^b	0.0076±0.00045	0.0013±0.00031 ^b	0.008±0.00042	0.002±0.00042	0.0087±0.00031	0.003±0.00011	0.01±0.00076 ^a	0.005±0.00011	0.016±0.00082 ^c	0.016±0.00011	0.03±0.00011	0.034±0.00011	0.035±0.00012	0.033±0.00054
Lmx1b	0.009±0.00053	0.0016±0.00032	0.01±0.00032	0.002±0.00045 ^b	0.011±0.00041	0.0025±0.00054 ^b	0.012±0.00021	0.003±0.00013 ^b	0.015±0.00037	0.0039±0.00011 ^{ab}	0.02±0.00042	0.006±0.0004	0.022±0.00054 ^a	0.009±0.00012	0.032±0.00012	0.028±0.00011	0.037±0.00011	0.037±0.0005
TH	0.0086±0.00012	0.001±0.00064 ^b	0.0114±0.00012	0.00213±0.00074 ^b	0.0111±0.00012	0.00218±0.00054 ^b	0.0134±0.00012	0.00262±0.00032 ^b	0.01453±0.00012	0.00308±0.00011 ^{ab}	0.0147±0.00012	0.00351±0.00015 ^a	0.015±0.00012	0.00599±0.00012	0.0281±0.00012	0.02612±0.00012	0.0275±0.00012	0.0262±0.00044
DAT	0.0122±0.00012	0.0018±0.00043 ^b	0.0164±0.00012	0.0021±0.00044 ^b	0.0183±0.00012	0.00248±0.00012	0.0208±0.00012	0.00384±0.00011 ^b	0.0213±0.00012	0.0047±0.00012	0.0215±0.00012	0.005±0.00012	0.0237±0.00012	0.00613±0.00012	0.03821±0.00012	0.03521±0.00012	0.0392±0.00012	0.0378±0.00012

Supplementary Table S6 — Regulation of gene expression at different time points by post injury of MPP⁺ (Day1-Day87)
with a fold change >1.0

Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day87 Male	Day87 Female
-7.4	-7.5	-6.3	-6.0	-5.4	-3.6	-2.7	-1.1	-0.9
-6.0	-5.8	-3.5	-3.4	-2.7	-2.5	-2.3	-1.0	-1.0
-6.5	-6.3	-5.8	-4.0	-2.9	-2.0	-1.3	-0.9	-1.1
-5.6	-5.0	-4.4	-4.0	-3.8	-3.3	-2.4	-1.1	-1.0
-7.5	-8.0	-4.3	-2.8	-2.5	-2.6	-2.3	-1.1	-1.3
-4.8	-5.0	-4.0	-2.8	-2.4	-2.2	-2.1	-1.1	-1.1
-6.0	-4.8	-4.7	-3.9	-3.7	-3.1	-3.0	-1.3	-1.1
-5.3	-5.8	-3.3	-3.1	-3.0	-2.7	-2.6	-1.1	-1.2
-8.3	-5.4	-5.1	-5.1	-4.7	-4.2	-2.5	-1.1	-1.0
-6.8	-7.8	-7.4	-5.4	-4.5	-4.3	-3.9	-1.1	-1.0