# Sex and Steroid Specific Affects on Neural Stem Cell Biology

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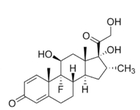
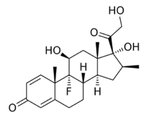
## Introduction

Introduction:

- Premature birth is a major risk factors for infants, as it is often associated with Respiratory Distress Syndrome (RDS) and intraventricular hemorrhage (IVH). These are combated clinically with the use of synthetic Glucocorticosteroids (sGCs) prenatally via administration to the mother.
- These sGCs function to expedite development of the lung and increase survival for premature infants at risk for RDS and IVH, but their prenatally use has also been associated with later neurological defects[1]. Furthermore, outcomes from sGCs use is sex dependents, indicating sex specific differences in the response to sGCs[1].
- The two most commonly used sGCs in the prenatal and postnatal periods are Dexamethasone (Dex) and Betamethasone (Beta) . These molecules are very chemically similar (shown below).

Dex  Beta 

- While Dex has been shown to decrease the risk of IVH in some studies[2,3], Dex use has also been implicated in the later development of neurological deficits[4], and the use of sGCs generally has been associated with defects in neuronal migration[5]. Relatively little work has been performed to compare the effects of Dex vs Beta on neurological outcome, and our work aims to explore how these sGCs induce distinct changes in the transcriptome, in neural stem cell biology, and adult function.

**Hypothesis**

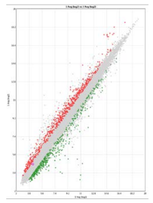
Previous studies suggest that male and female MNSCs respond differently to Dex[1]. We propose to compare the transcriptome in male and female MNSCs, and to identify Sex and steroid specific response to the corticosteroids, Dex and Beta. We further propose that Dex and Beta will have differential affects on MNSC biology.

## Methodology

- MNSCs were prepared from E14.5 mouse cortices and RNA isolated at P2 after 4 hours of Dex or Vehicle stimulation. Affymetrix GeneChip array analysis was performed to identify whole genome transcriptional changes in male versus female MNSCs. The transcriptome analysis console (TAC) was used to analyze genes that were either upregulated or downregulated respectively to the treatment of control, Dex, and Beta treated samples. Significance was set at $p < 0.05$ and fold change >1.5.
- Quantitative Polymerase Chain Reactions (PCRs) were performed to confirm the results of the Affymetrix gene chip. RNA extracted from cells treated with Ethanol, Beta, or Dex was converted to cDNA using the Thermo Fisher Scientific High Capacity RNA-to-cDNA kit. qPCRs were performed with primers to genes of interest and compared against GAPDH. Genes and GAPDH are run in duplicate and analyzed using the delta CT method.
- For proliferation and differentiation experiments 20,000 MNSCs were plated per well in a 96 well plate. 24 hours later, in one set of MNSCs were treated with $10^{-7}$ M Ethanol (vehicle), Dex, and Beta overnight, then treated with differentiation media and grown for 3 days. In other set, cells were allowed to grow in proliferation media for 24 hours, then proliferation media was replaced with differentiation media containing $10^{-7}$ M Ethanol, Dex, and Beta for 2 days. Cells from both plates were washed with PBS and fixed with paraformaldehyde. Cells were immunostained for different cell population specific antibodies including: Nestin (stem cells), CNpase (oligodendrocytes), and Beta-3-tubulin (neurons). Images were captured using the EVOS FL auto-imaging system. Cells were counted in Photoshop's, means and statistical significance calculated using a T-Test.

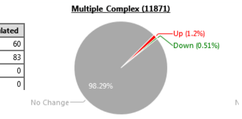
## Results

### 1. Comparison of the Transcriptome in Male and Female MNSCs

A 

B

| Group | Total | Passed Filter | Up-Regulated | Down-Regulated |
|---|---|---|---|---|
| Multiple_Complex | 11871 | 203 | 143 | 60 |
| Coding | 10261 | 173 | 90 | 83 |
| Pseudogene | 14 | 1 | 1 | 0 |
| NonCoding | 60 | 0 | 0 | 0 |




A. Scatter plot representation of the basal differences in gene expression in female vs male MNSCs. Log2 fold differences in upregulated genes (red) and downregulated genes (green) are shown.
B. Summary data showing the number and type of sex specific gene changes.

### 2. Gene expression changes in Male vs Female MNSCs

Top Genes increased in Male Neural Stem Cells vs Female

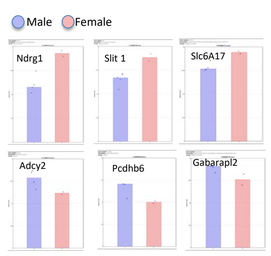
A

| Gene Symbol | P-val | Female Avg (log2) | Male Avg (log2) | Fold Change |
|---|---|---|---|---|
| Fstl1 | 0.0058 | 6.03 | 8.31 | -4.87 |
| Ltbp4 | 0.0009 | 6.08 | 8.38 | -4.94 |
| Rab26 | 0.0453 | 5.02 | 7.37 | -5.1 |
| Col18a1 | 0.0326 | 5.02 | 7.39 | -5.19 |
| Ankrd37 | 1.93E-05 | 5.56 | 7.96 | -5.26 |
| Ndrg1 | 0.0008 | 4.03 | 6.49 | -5.52 |
| Aebp1 | 0.049 | 6.87 | 9.4 | -5.79 |
| Pfkp | 0.0061 | 6.68 | 9.33 | -6.26 |
| Cox6b2 | 0.0474 | 9.41 | 12.1 | -6.42 |
| Anxa2 | 0.0349 | 5.1 | 8.09 | -7.94 |
| Cspg4 | 0.0009 | 7.88 | 10.87 | -7.95 |
| Dhh | 0.0113 | 4.32 | 7.48 | -8.97 |
| Mcam | 0.0214 | 5.51 | 8.81 | -9.83 |
| Nr2f2 | 0.0403 | 6.32 | 9.85 | -11.48 |
| Fbln2 | 0.0402 | 8.29 | 12.03 | -13.39 |
| Adamts13 | 0.0382 | 5.17 | 9.21 | -16.42 |
| Slc16a3 | 2.27E-06 | 3.88 | 8.13 | -19.1 |
| Kdm5d | 2.23E-09 | 3.76 | 8.4 | -24.83 |
| Angpt2 | 0.0406 | 5.16 | 10.51 | -40.7 |
| Ddx3y | 1.23E-09 | 4.73 | 11.23 | -90.26 |
| Uty | 4.24E-10 | 3.6 | 10.96 | -164.56 |
| Eif2s3y | 2.31E-12 | 3.15 | 10.89 | -214.72 |

Top Genes Increased in Female MNSCs vs Male

B

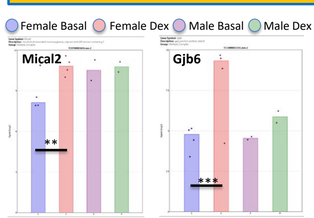
| Gene Symbol | P-val | Female Avg (log2) | Male Avg (log2) | Fold Change |
|---|---|---|---|---|
| Hist1h1c | 0.0174 | 12.28 | 9.55 | 6.6 |
| Pnp | 0.0197 | 12.01 | 9.54 | 5.54 |
| Pip5k1b | 0.0158 | 10.19 | 7.86 | 5.04 |
| Pacrg | 0.0059 | 9.84 | 7.54 | 4.9 |
| Pla2g4a | 0.0417 | 7.84 | 5.64 | 4.6 |
| Pcdhb6 | 0.0246 | 7.07 | 5.01 | 4.16 |
| Gsten7 | 0.0135 | 11.99 | 9.97 | 4.06 |
| Nacc2 | 0.0321 | 6.71 | 4.76 | 3.85 |
| Gabarapl2 | 0.0051 | 11.83 | 9.89 | 3.83 |
| Mgst1 | 0.0287 | 14.21 | 12.28 | 3.82 |
| Ndp | 0.0324 | 11.38 | 9.45 | 3.82 |
| Trigk | 0.0183 | 8.32 | 6.39 | 3.81 |
| Rab4a | 0.0115 | 10.68 | 8.76 | 3.78 |
| Socs2 | 0.0269 | 9.26 | 7.39 | 3.64 |
| Gpx8 | 0.0249 | 12.15 | 10.29 | 3.61 |
| Ccbl2 | 0.0076 | 9.05 | 7.21 | 3.59 |
| Stard9 | 0.0227 | 10.44 | 8.6 | 3.58 |
| Pex11a | 0.0434 | 8.31 | 6.48 | 3.55 |
| Rnd1 | 0.0056 | 8.38 | 6.59 | 3.45 |
| Smim4 | 0.0278 | 9.54 | 7.76 | 3.42 |
| Zrsr1 | 0.0352 | 8.78 | 7.01 | 3.42 |

C 


All at p< 0.05

A/B. Tables showing the sex specific fold change in gene expression in NSC. Select genes are shown in the tables (A,B) and select gene expression values are shown in C.
C. N-Myc Downstream Regulated 1, Slit 1, and Solute Carrier 6A17 (neurotransmitter reuptake) are all increased in male MNSCs as compared to female. Adenylate cyclase 2, Protocadherin Beta 6, and GABA-A Receptor Associated protein-like 2 are all increased in female MNSCs compared to male

### 3. Dex response of male vs female MNSCs



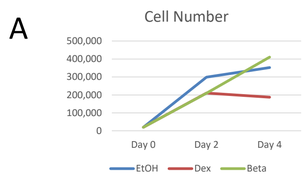

3: Sample log2 fold signal sequences in Mical2 (Microtubule Associated Monooxygenase) and Gjb6 (Gap Junction Protein Beta 6) in Males vs Females MNSC in response to Dex. P * <0.05, ** <0.01, *** < 0.001

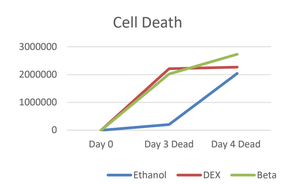
### 4. Dex vs Beta response female MNSCs


4: PPBP (Proplatelet Basic Protein), SYPL2 (Synaptophysin like-2), and SYT17 (Synaptotagmin XVII) respond differently to Dex vs Beta in female MNSCs. P * <0.05, ** <0.01, *** < 0.001

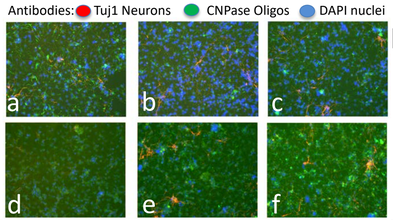
## Results

### 5. Comparison of the Biological response of MNSCs to Dex vs Beta

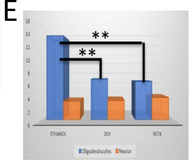
A 

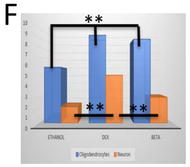

B 


Antibodies: Tuj1 Neurons CNPase Oligos DAPI nuclei

C 


D 


E 


F 


P = * <0.05, **<0.01

Comparison of the biological response of MNSCs to Dex or Beta stimulation.

A. MNSCs proliferation: Dex and Beta show different Proliferative responses. While Beta and Dex initially demonstrates reduced proliferation compared to Ethanol (EtOH), Dex treated MNSC cease to proliferation after 2 days.
B. Cell death in differentiating cells with EtOH vs Dex vs Beta: Increased death is seen with both Dex and Beta as compared to Ethanol.
C. When proliferating MNSCs are exposed to Dex(b) or Beta(c) the proportion of oligodendrocytes decreases without an alteration in neurons, compared to EtOH(a) (Quantified in E).
D. If MNSCs are exposed to sGC while differentiating, both Dex(e) and Beta(f) induce oligodendrocyte differentiation but Dex induced more neurons compared to Beta(f) and EtOH(d) (Quantified in F).

## Summary/Conclusion

- Basally, male and female MNSCs show significant differences in gene expression. Some of the most significant changes are in genes related to neuronal growth, differentiation, and neurotransmitter regulation.
- Male and female MNSC show significant differences in response to Dex. Genes related to neuronal growth, nonsyndromic deafness, and IVH have shown differential response to Dex between males and females. Interestingly the GJB6 gene that encodes a gap junction is upregulated by Dex in females but not in males. This could play a role in the pathology of IVH.
- Dex and Beta induce different genes to different levels, including several genes related to the fetal brain and blood vessel growth/hematopoiesis.
- Dex and Beta have anti-proliferative properties in MNSCs but Dex has more potent anti-proliferative properties after 48 hours. Proliferating MNSCs exposed to Dex and Beta produce fewer oligodendrocytes than Ethanol treated MNSCs suggesting the sGC reprograms stem cells to an oligodendrocyte lineage.
- Differentiating MNSCs exposed to Dex and Beta show an increased number and more mature oligodendrocytes that Ethanol. Differentiating MNSCs showed increased neurons when exposed to Dex.

## References

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