Repeated ropinirole treatment resulting in recovery of sensorimotor gating induces ΔFosB in mouse nucleus accumbens neurons that co-express D_1 and D_3 dopamine receptors, but not D_2 receptors.

Kimberly T. Meyers[1,2], Amanda M. Maple[2], Deena M. Walker[3], Michael E. Cahill[3], Amelia L. Gallitano[1,2,4], Ella M. Nikulina[2], Eric J. Nestler[3], Ronald P. Hammer, Jr.[1,2,4,5]

[1]Interdisciplinary Graduate Program in Neuroscience, Arizona State Univ., Tempe, AZ; [2]Basic Medical Sciences, Univ. Arizona College of Medicine, Phoenix, AZ; [3]Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY; [4]Psychiatry, Univ. Arizona College of Medicine, Phoenix, AZ; [5]Pharmacology, Univ. Arizona College of Medicine, Tucson, AZ.

BACKGROUND

- Prepulse inhibition (PPI), a behavioral task indicative of sensorimotor gating deficits in schizophrenia, is reduced by acute treatment with a D_2-like receptor agonist (Martinez, Ellison, Geyer, & Swerdlow, 1999), or by its local administration in the nucleus accumbens (NAc) (Wan & Swerdlow, 1993).
- By contrast, repeated treatment with quinpirole or ropinirole causes PPI recovery lasting up to four weeks after termination of treatment in rats (Berger, Green, Siegel, Nestler, & Hammer, 2011; Culm, Lugo-Escobar, Hope, & Hammer, 2004).
- Repeated quinpirole treatment induces activation of the cyclic AMP (cAMP) pathway as evidenced by an increase of cAMP-dependent protein kinase (PKA) activity and CREB phosphorylation in the NAc (Culm & Hammer, 2004).
- We have demonstrated that repeated quinpirole treatment induces prolonged expression of ΔFosB, a truncated splice variant of FosB protein, in the NAc. ΔFosB is induced by chronic activation of cAMP signaling, has extended stability, and is implicated in long-term behavioral adaptation (Nestler, Barrot, & Self, 2001).
- In the NAc, ΔFosB induction has different behavioral effects depending on whether it is present in D_1-like or D_2-like dopamine receptor-expressing neurons (Grueter, Robison, Neve, Nestler, & Malenka, 2013).
- **Therefore, we investigated whether, and in which NAc neurons, ΔFosB was expressed after repeated ropinirole treatment.**

METHODS

Animals and Drug Treatment

Adult male BAC transgenic mice that express either tdTomato in striatal neurons containing dopamine 1 receptor cell subtype (D1R-tdTomato) or eGFP in striatal neurons containing dopamine 2 receptor cell subtype (D2R-EGFP) (Gong et al., 2003) were treated daily with sterile saline vehicle or ropinirole HCl (0.1 mg/kg, ip) for 28 days.

Immunohistochemistry

Seven days after termination of drug treatment, mice were anesthetized and perfused with 4% buffered paraformaldehyde. Striatal brain sections (40 µm) were collected and stored in cryopreservative at 4°C prior to processing. Sections were washed three times in 0.05 M potassium phosphate-buffered saline (KPBS, pH 7.4), and incubated in 5% normal goat serum/0.05 m KPBS/0.4% Triton X (NGS/KPBS/TX) for 60 min at room temperature.

To determine ΔFosB labeling, primary antibody raised against the N-terminal region of FosB (SC-48; Santa Cruz Biotechnology, Santa Cruz, CA) was used at a dilution of 1: 12,000 in (NGS/KPBS/TX). This antibody recognizes 32–37 kDa proteins corresponding to the molecular weight of ΔFosB-like proteins, as well as full-length FosB (Perrotti et al., 2004). Primary antibody raised against the dopamine 3 receptor subtype (D_3R; (SC-9114; Santa Cruz Biotechnology, Santa Cruz, CA was used at a dilution of 1: 20 in NGS/KPBS/TX to determine D_3R labeling.

Immunohistochemical analysis

Brain sections on slides were analyzed at 20X magnification using a Zeiss microscope and Image J Software. A modified stereological approach was employed to randomly assess neuronal labeling density. The total number of labeled neurons was analyzed using two-way ANOVA.

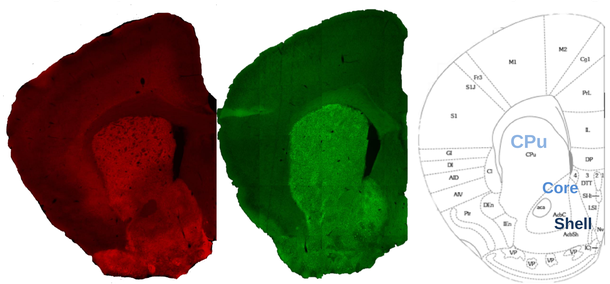

Figure 1. D1R-tdTomato (left) and D2-eGFP (middle) mouse striatal hemisections, and brain regions in which ΔFosB or D_3R labeling was assessed (right).
Abbreviations:
CPu = caudatoputamen
Core = NAc Core
Shell = NAc Shell

ACKNOWLEDGEMENTS

Supported by USPHS Awards MH073930 (RPH) and DA026451 (EMN); NIH T32DA007135 (DMW), R01MH097803 (ALG), P50MH096890 (EJN); Science Foundation of Arizona Bisgrove Scholarship (AMM). Ropinirole was generously provided by the NIMH Chemical Synthesis and Drug Supply Program.

RESULTS: D1-tdTamato mice

- **Striatal distribution of D_1R and D_3R was unaffected by repeated drug treatment.**

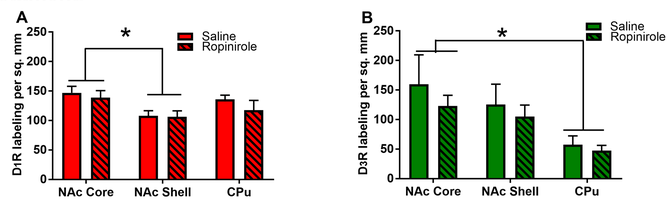

Figure 2. A) D_1R and B) D_3R labeling per sq. mm (mean ± SEM) **in striatal regions after 28 days of treatment.** No significant effect of drug treatment or interaction between brain region and drug treatment was observed. Each treatment group contained 5 mice. * - Significant main effect of brain region ($p < 0.05$).

- **Repeated ropinirole treatment induced ΔFosB in the NAc core and shell, but not in the CPu of D_1R-tdTomato mice.**

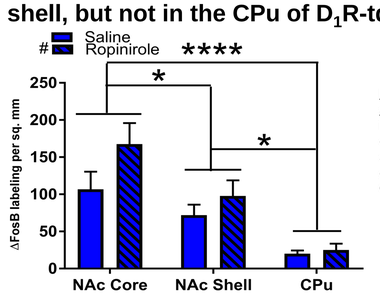

Figure 3. ΔFosB labeling per sq. mm (mean ± SEM) in striatal regions after 28 days of treatment. No significant interaction between brain region and drug treatment was observed. Significant main effects of brain region ($p < 0.0001$) and drug treatment ($p \leq 0.05$) were observed. Each treatment group contained 5 mice. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, # $p \leq 0.05$ compared to saline.

- **After repeated ropinirole treatment, ΔFosB expression was co-localized in both D_1R- and D_3R-labeled NAc neurons with the greatest effect in NAc core neurons containing both D_1R and D_3R.**

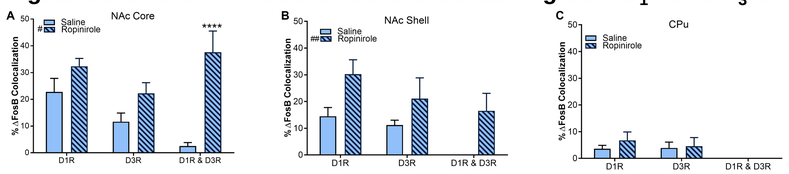

Figure 4. Percentage of ΔFosB co-localization (mean ± SEM) in A) D_1R-,B) D_3R-, and C) D_1R & D_3R-labeled cells in the NAc core, shell, and CPu after 28 days of treatment. In the NAc core, a significant main effect of drug treatment ($p < 0.0001$) and significant interaction between dopamine receptor subtype and drug treatment ($p < 0.05$) were observed. In the NAc shell, significant main effects of dopamine receptor subtype ($p < 0.05$) and drug treatment ($p < 0.01$) were present. In the CPu, no significant effect of dopamine receptor subtype or drug treatment was present. Each treatment group contained 5 mice. **** $p < 0.0001$.

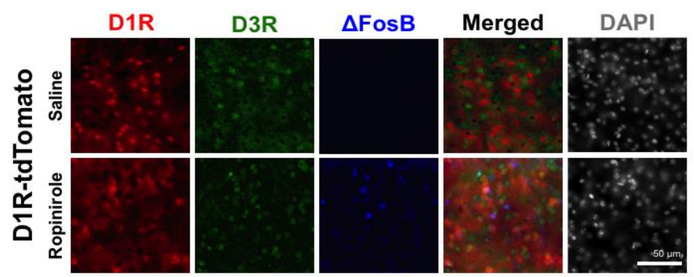

Figure 5: D_1R, D_3R, ΔFosB, and DAPI immunolabeled cells in the NAc core. *40X magnification*

RESULTS: D2-eGFP mice

- **Striatal distribution of D_3R, but not D_2R was altered by repeated drug treatment.**

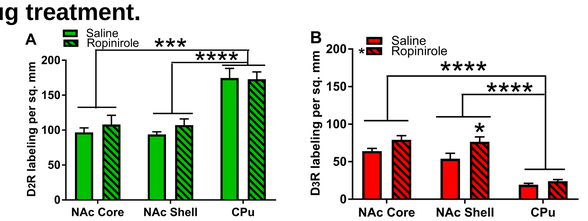

Figure 6. D_2R and D_3R labeling per sq. mm (mean ± SEM) in striatal regions after 28 days of treatment. No significant main effect of drug treatment or interaction between brain region and drug treatment was observed in D_2R-expressing neurons. However, a significant main effect of drug treatment was present in D_3R-expressing neurons in the NAc shell. Ropinirole treated group contained 8 mice; saline treated group contained 6 mice. *($p < 0.01$). Significant main effect of brain region **** $p < 0.0001$, and *** $p < 0.001$.

- **Repeated ropinirole treatment induced ΔFosB in NAc core and shell, but not in the CPu of D_2-eGFP mice.**

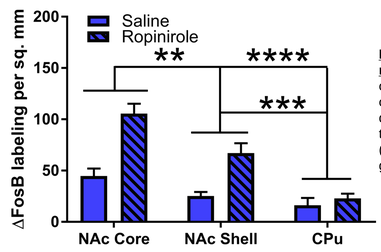

Figure 7. ΔFosB labeling per sq. mm (mean ± SEM) **in striatal regions after 28 days of repeated treatment.** Significant main effects of brain region ($p < 0.0001$) and drug treatment ($p < 0.0001$) were observed. A significant interaction between brain region and ropinirole drug treatment was observed: ** ($p < 0.01$) between the NAc core and the NAc shell; **** ($p < 0.0001$) between the NAc core and the CPu; *** ($p < 0.001$) between the NAc shell and the CPu. Ropinirole treated group contained 8 mice; saline treated group contained 6 mice.

- **After repeated ropinirole treatment, co-localization of ΔFosB expression was present in D_3R-, but not D_2R- labeled NAc neurons.**

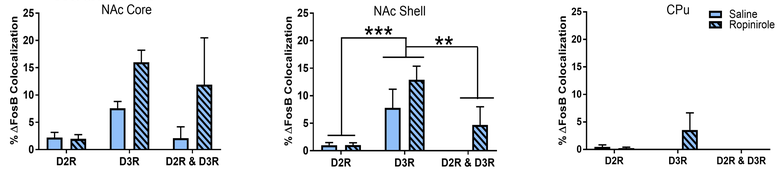

Figure 8. Percentage of ΔFosB co-localization (mean ± SEM) **in D_2R-, D_3R-, and D_2R & D_3R- labeled cells in the NAc core, shell, and CPu after 28 days of treatment.** In the NAc shell, a significant interaction between dopamine receptor subtype and drug treatment was observed ($p < 0.001$). In the NAc core and the CPu, no significant main effect of dopamine receptor subtype or drug treatment was observed. Ropinirole treated group contained 8 mice; saline treated group contained 6 mice.

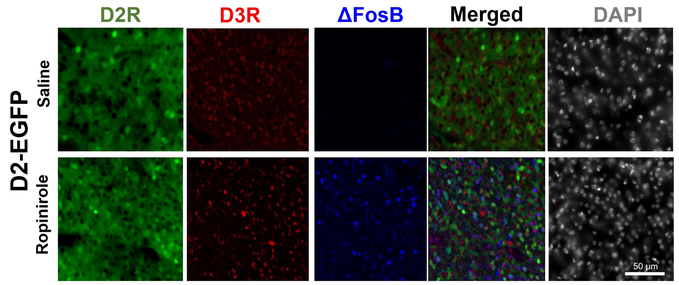

Figure 9: D_1R, D_3R, ΔFosB, and DAPI immunolabeled cells in the NAc core. *40X magnification*

SUMMARY AND CONCLUSIONS

- Repeated ropinirole treatment induced ΔFosB selectively in NAc neurons of both D_1R-tdTomato and D_2-eGFP mice, confirming our prior results.
- Repeated ropinirole treatment significantly increased the percentage of ΔFosB only in NAc neurons which co-expressed D_1 and D_3 receptors.
- **Repeated activation by ropinirole of heterodimeric D_1 / D_3 receptors in NAc neurons could drive D_1 receptor-coupled signaling, which could underlie increased phosphoCREB and ΔFosB expression required for PPI recover**

REFERENCES

Berger, A. K., Green, T., Siegel, S. J., Nestler, E. J., & Hammer, R. P., Jr. (2011). cAMP response element binding protein phosphorylation in nucleus accumbens underlies sustained recovery of sensorimotor gating following repeated D-like receptor agonist treatment in rats. *Biol Psychiatry, 69*(3), 288-294. doi:S0006-3223(10)00940-6 [pii]10.1016/j.biopsych.2010.08.032

Culm, K. E., & Hammer, R. P., Jr. (2004). Recovery of sensorimotor gating without G protein adaptation after repeated D2-like dopamine receptor agonist treatment in rats. *J Pharmacol Exp Ther, 308*(2), 487-494. doi:10.1124/jpet.103.057158jpet.103.057158 [pii]

Culm, K. E., Lugo-Escobar, N., Hope, B. T., & Hammer, R. P., Jr. (2004). Repeated quinpirole treatment increases cAMP-dependent protein kinase activity and CREB phosphorylation in nucleus accumbens and reverses quinpirole-induced sensorimotor gating deficits in rats. *Neuropsychopharmacology, 29*(10), 1823-1830. doi:10.1038/sj.npp.1300483130048 3 [pii]

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., . . . Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature, 425*(6961), 917-925. doi:10.1038/nature02033

Grueter, B. A., Robison, A. J., Neve, R. L., Nestler, E. J., & Malenka, R. C. (2013). FosB differentially modulates nucleus accumbens direct and indirect pathway function. *Proc Natl Acad Sci U S A, 110*(5), 1923-1928. doi:10.1073/pnas.1221742110

Martinez, Z. A., Ellison, G. D., Geyer, M. A., & Swerdlow, N. R. (1999). Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. *Psychopharmacology (Berl), 142*(3), 253-260.

Nestler, E. J., Barrot, M., & Self, D. W. (2001). DeltaFosB: a sustained molecular switch for addiction. *Proc Natl Acad Sci U S A, 98*(20), 11042-11046. doi:10.1073/pnas.191352698898/20/11042 [pii]

Perrotti, L. I., Hadeishi, Y., Ulery, P. G., Barrot, M., Monteggia, L., Duman, R. S., & Nestler, E. J. (2004). Induction of deltaFosB in reward-related brain structures after chronic stress. *J Neurosci, 24*(47), 10594-10602. doi:24/47/10594 [pii]10.1523/JNEUROSCI.2542-04.2004

Wan, F. J., & Swerdlow, N. R. (1993). Intra-accumbens infusion of quinpirole impairs sensorimotor gating of acoustic startle in rats. *Psychopharmacology (Berl), 113*(1), 103-109.