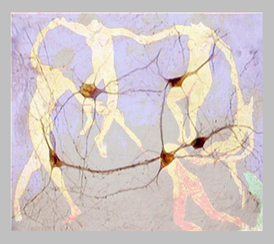

Abnormal development of monoaminergic neurons is implicated in fluctuations of manic- and depressive-like behavior and bipolar disorder

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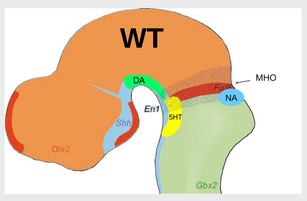
INTRODUCTION

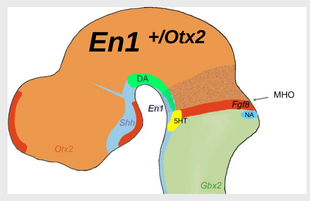
Previously, we demonstrated that the transcription factor *Otx2* activates a genetic network including *Wnt1* and *Gsk3* that is essential in determining the number of monoaminergic neurons generated in the brain during development and present later in adulthood (Brodski et al., 2003).

Mouse mutants overexpressing *Otx2* are hyperactive, show an increase of dopaminergic neurons and a decrease of serotonergic and noradrenergic neurons. These changes are mediated by alterations in the *Wnt1*, *Bmp* and *Gsk3-beta* pathway.

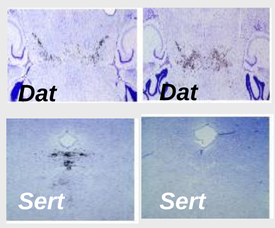
Based on data suggesting polymorphisms in the OTX2 gene as a potential risk factor for bipolar disorder (Sabunciyan et al., 2007), we tested the hypothesis that Otx2 overexpressing animals recapitulate aspects of bipolar disorder.

Adult Otx2 mutants show alterations in monoaminergic neurons





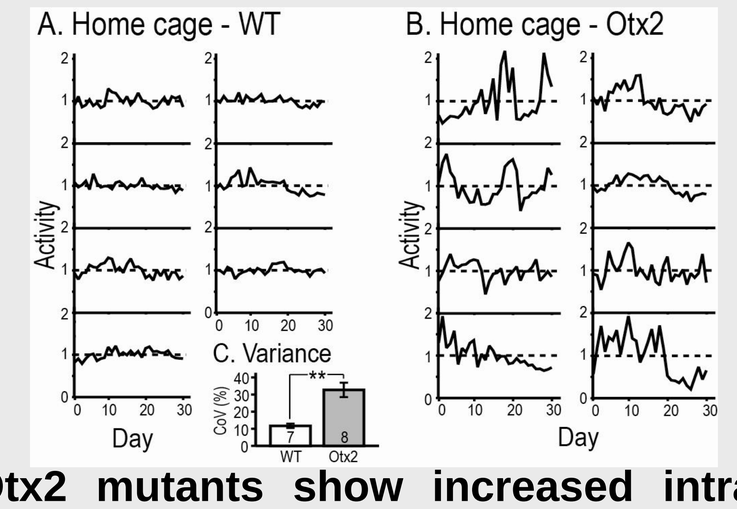
Wildtype *En1 +/Otx2*



Dopaminergic neurons ↑ **15%**

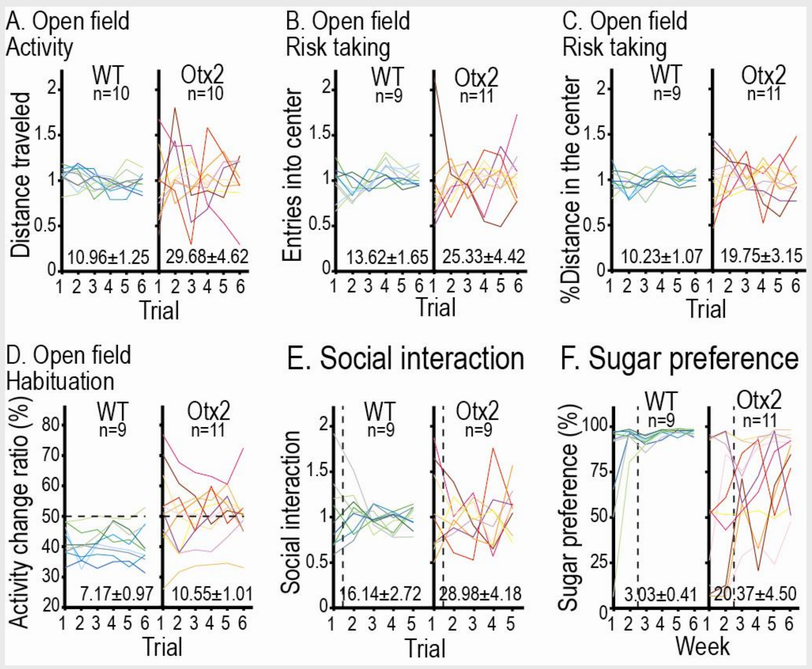
Serotonergic neurons ↓ **25%**

RESULTS



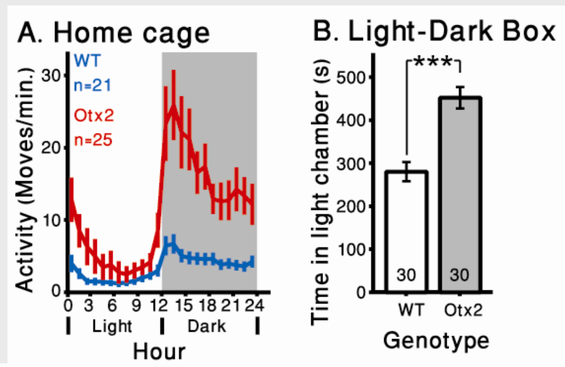
Otx2 mutants show increased intra-individual fluctuations in spontaneous locomotor activity in their home cage

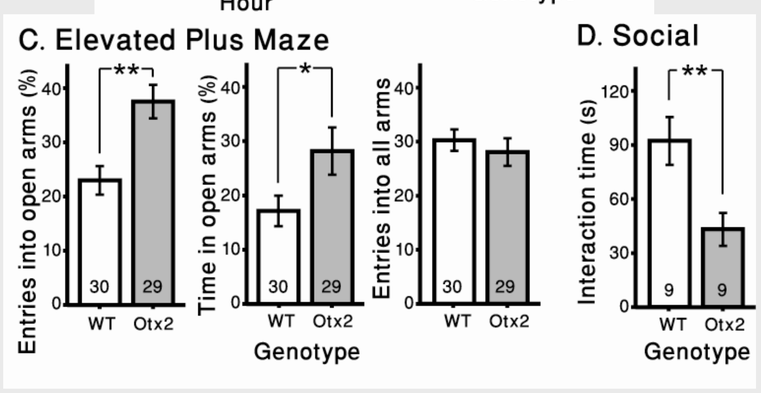
Animals were studied for 30 days under normal conditions. Average activity for every 24 hours was measured, normalized to average activity level of the animal during the whole period and shown as a function of day in separate graphs for (**A**) WTs and (**B**) Otx2 mutants. Individual animals are represented by different graphs. (**C**) Otx2 mutants show increased intra-individual fluctuation as indicated by increased coefficient of variance (t_{18}=4.468, p=0.001), as calculated by dividing the standard deviation by the mean value.



Otx2 mutants fluctuate more in various behavioral paradigms.

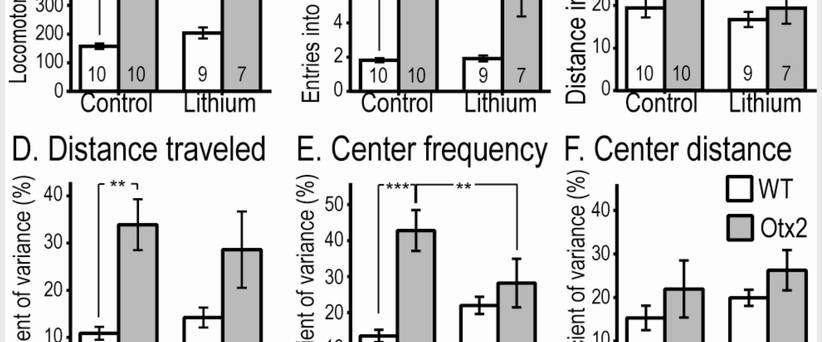
Animal activity was recorded for one hour in the open field for six consecutive trials during 21 days and parameters for the center of the open field analyzed **(A-D)**. Otx2 animals showed increased coefficient of variance for (**A**) activity levels (t_{18}=3.029, p=0.007), (**B**) the number of entries into the center (t_{18}=2.482, p=0.028), and (**C**) the percentage of the total distance they traveled in the center (t_{18}=2.864, p=0.014). Coefficient of variance for Otx2 mutants was also increased for (**D**) activity-change ratios (t_{18}=2.38, p=0.027). In the social interaction test, animals of the same genotype were recorded for five minutes. Fluctuation in time animals interacted was calculated for four trials, after one initial habituation trial. Mutants (**E**) showed an increased coefficient of variance for the time they interacted (t_{16}=2.572, p=0.020). Variance in sweet preference was measured during the course of four weeks, after two weeks of habituation. As quantified by an increase in the coefficient of variance mutants showed more fluctuations over time for (**F**) sucrose preference (t_{18}=3.458, p=0.003).





Otx2 mutants are hyperactive and they show more risk taking behavior

Animals were implanted with transmitters and their activity was monitored in hour bins for five days. (**A**) Mutants showed increased activity throughout the dark phase and also in the first two and the last hours of the light phase ($^{Genotype}F_{1,44}$=17.152 p<0.001). (**B**) *Otx2* animals spent significantly more time in the light compartment In the light dark box than WTs (t_{18}=5.149, p<0.001). In the elevated plus maze, mutants showed (**C**) more entries into (t_{18}=3.583, p=0.001) and time spent (t_{18}=2.149, p=0.036) in the open arms compared to controls, while number of entries into all arms didn't differ between groups (t_{18}=0.684, p=0.497). (**D**) Sociability of mutants was decreased compared to WTs (t_{16}=2.945, p=0.011)

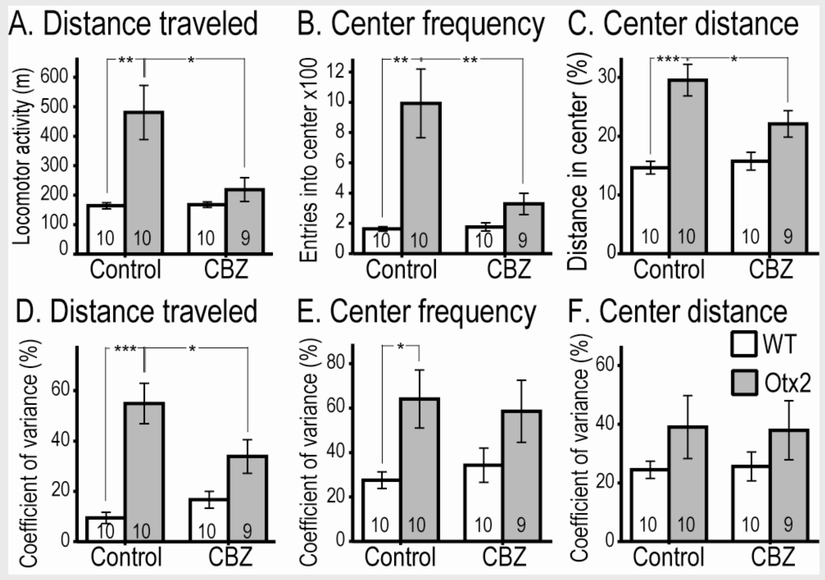


Acute olanzapine treatment improves manic-like behavior of mutants

In the elevated plus maze, OZP treatment selectively reduces (**A**) the number of entries into open arms in Otx2 mutants ($^{Genotype*Treatment}F_{1,36}$=4.663, p=0.038). OZP decreases the number of entries into all arms of the EPM ($^{Treatment}F_{1,36}$=6.065, p=0.019) with no significant difference between genotypes ($^{Genotype*Treatment}F_{1,36}$=0.335, p=0.566). (**B**) In the light-dark box, treated mutants show a decrease in time spent in the light compartment ($^{Genotype*Treatment}F_{1,36}$=5.666, p=0.005). (**C**) OZP reduces the activity of both controls and mutants in the open field ($^{Treatment}F_{1,36}$=4.834, p=0.034), without difference in the effect between groups ($^{GenotypexTreatment}F_{1,36}$=0.588, p=0.448).

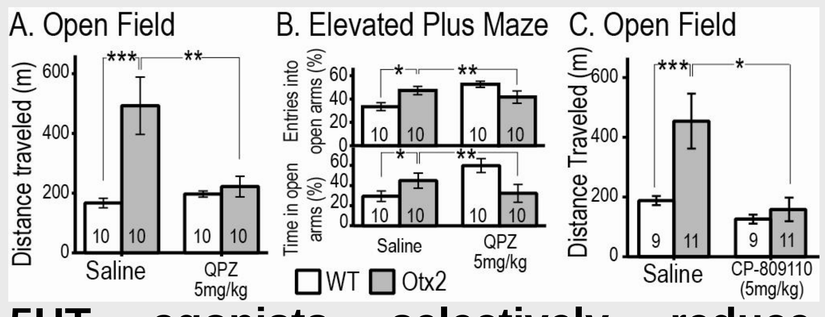
Lithium improves some of the altered behavioral parameters in Otx2 mutants

All animals were monitored during a one hour open field trial for six consecutive trials during three weeks. Lithium didn't significantly reduce (**A**) locomotor activity ($^{Genotype*Treatment}F_{1,31}$=0.005, p=0.942) and (**B**) the number of entries into the center of the open field ($^{Genotype*Treatment}F_{1,31}$=1.794, p=0.19), while for (**C**) distance traveled in the center a significant specific reduction was observed in mutants ($^{Genotype*Treatment}F_{1,31}$=4.291, p=0.047). (**D**) Lithium treatment didn't affect coefficient of variance for locomotor activity in mutants ($^{Genotype*Treatment}F_{1,31}$=0.856, p=0.362), while reducing it selectively for the (**E**) frequency of entries into the center ($^{Genotype*Treatment}F_{1,31}$=6.544, p=0.016). (**F**) There was no interaction between lithium treatment and genotype in coefficient of variance for distance traveled in the center ($^{Genotype*Treatment}F_{1,31}$=0.001, p=0.947).



Carbamazepine normalize behavioral alterations in Otx2 mutants

All animals were monitored during a one hour open field trial for four consecutive trials during two weeks. Carbamazepine selectively reduced (**A**) locomotor activity ($^{Genotype*Treatment}F_{1,35}$=6.732, p=0.014); (**B**) frequency of entries ($^{Genotype*Treatment}F_{1,35}$=7.679, p=0.009) and (**C**) distance traveled in the center ($^{Genotype*Treatment}F_{1,35}$=4.696, p=0.037) of the open field. Coefficient of variance was significantly reduced in mutants for their (**D**) locomotor activity ($^{Genotype*Treatment}F_{1,35}$=6.462, p=0.016), while not observed for (**E**) frequency of entries ($^{Genotype*Treatment}F_{1,35}$=0.358, p=0.553) and (**F**) distance traveled in the center of the open field ($^{Genotype*Treatment}F_{1,35}$=0.021, p=0.887).



5HT agonists selectively reduce hyperactivity in Otx2 mutants

During one hour testing in the open field 15 minutes after IP injection of saline, quipazine, or CP-809110 (**A**) quipazine specifically decreased distance traveled in the open field for mutants ($^{Genotype*Treatment}F_{1,36}$=8.429, p=0.006). (**B**) There was a significant interaction between genotype and quipazine treatment in number of entries into (Fig9B; $^{Genotype*Treatment}F_{1,36}$=11.534, p=0.002) and time spent (Fig9B; $^{Genotype*Treatment}F_{1,36}$=9.286, p=0.005) in open arms of EPM with no such interaction in the number of entries into all arms ($^{Genotype*Treatment}F_{1,36}$=1.352,

Gene List	Number of genes	Pval. BPD	Pval. SCZ	Pval MD
Dopaminergic specification	34	0.011 0.033*	1	1
Dopaminergic specification (Hegarty et. al)	26	0.025 0.042*	1	1
Serotonergic specification	19	0.028	1	1

* FDR corrected p values; **BPD**, bipolar disorder; **SCZ**, schizophrenia; **MD**, major depression

Genes controlling the specification of 5HT and DA neurons are associated with bipolar disorder in humans

Using INRICH software we tested whether sequence variants in genes involved in the specification of monoaminergic neurons are involved in psychiatric disorders. The dopaminergic specification pathway showed a significant global enrichment for BD (p=0.011), whereby 10 out of 34 genes were in associated intervals. For the serotonergic specification pathway we also found significant global enrichment (p=0.028), whereby 4 out of 19 genes were found in associated intervals. Recently, a list compiling all mouse mutants that show alterations in the development of dopaminergic neurons has been reported (Hegarty et al., 2013). We found that genes controlling the specification of DA neurons showed significant global enrichment (p=0.025).

SUMMARY

- Otx2 mutants show increased spontaneous fluctuations in their behavior.
- Otx2 mutants are hyperactive and show increased risk taking behavior.
- Olanzapine, lithium, carbamazepine and serotonin receptor agonists reverse behavioral alterations of Otx2 mutants.
- Sequence variants in genes controlling the development of monoaminergic neurons are associated with BPD

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