Microtubule Stabilizer Ameliorates Synaptic Function and Behavior in a Mouse Model for Schizophrenia

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Running head: Epothilone D in STOP-null mice

Key words: microtubule, synaptic plasticity, epothilone, STOP protein, schizophrenia

Number of words in text: 4489

Number of words in abstract: 199

Number of figures: 5

Number of tables: 0

Summary

Background: Recent data suggest a role of cytoskeletal defects in schizophrenia. We have previously obtained an animal model which recapitulates features of schizophrenia, by disrupting in mice a gene coding for a microtubule-associated protein called STOP. STOP null mice display synaptic defects in glutamatergic neurons, hyper-dopaminergy and severe behavioral disorders. Synaptic and behavioral deficits are amended by neuroleptic treatment in STOP null mice, which provide an attractive model for test of new anti-psychotic agents. Here, we have examined the effects of a taxol related microtubule stabilizer, epothilone D. **Methods**: Mice were treated either with vehicle alone or with epothilone D. Treatment effects on synaptic function were assessed using electron-microscopy quantification of synaptic vesicle pools and electrophysiology in CA1 region of the hippocampus. Dopamine transmission was investigated using electro-chemical assays. Behavior was principally assessed using tests of maternal skills. **Results**: In STOP null mice, a treatment with epothilone D increases synaptic vesicle pools and ameliorates both short- and long-term forms of synaptic plasticity in glutamatergic neurons and has a dramatic beneficial effect on mouse behavior. **Conclusions**: A microtubule stabilizer can have a beneficial effect on synaptic function and on behavior, and this suggests new possibilities for the treatment of schizophrenia.

Introduction: Schizophrenia is a common and debilitating mental illness that exacts considerable human and economic costs (Schultz and Andreasen 1999). The origin of schizophrenia is still debated but current data favor a model in which schizophrenia arises from defects in neuronal connectivity, principally caused by synaptic alterations (Mirnics et al 2001; Owen et al 2005a; Owen et al 2005b). Recently, the protein coded by a gene known to be disrupted in a familial form of schizophrenia (DISC1) has been characterized and shown to be involved in a variety of interactions with microtubule-related organelles or proteins (Callicott et al 2005; Morris et al 2003), suggesting that connectivity disorders in schizophrenia can result from cytoskeletal alterations. Consistent with this hypothesis, we have obtained a genetic animal model, which displays a set of behavioral alterations and of neurotransmission defects reminiscent of schizophrenic disorders, by disrupting in mice the STOP gene, which encodes a microtubule-associated protein (Stable Tubule Only Polypeptide, STOP protein)(Bosc et al 2003; Bosc et al 1996; Guillaud et al 1998). STOP-null mice have an apparently normal brain anatomy but display a dramatic depletion of synaptic vesicle pools and severe impairments of synaptic plasticity in hippocampal glutamatergic neurons, indicating defects in the glutamatergic transmission (Andrieux et al 2002). STOP null mice also exhibit hyper-dopaminergy in the limbic system (Brun et al 2005). Such neurotransmission defects are consistent with the association of hypoglutamatergy and of hyper-dopaminergy, currently considered to be a landmark of schizophrenia (Frankle et al 2003), and are associated in STOP null mice with severe behavioral deficits, including a fragmentation of spontaneous activity, hyper-locomotor activity, anxiety-related disorders, signs of severe social withdrawal, dramatic perturbations

of maternal behavior and a dysfunction of sensory-gating mechanisms which is also observed in schizophrenic patients (Andrieux et al 2002; Fradley et al 2005).

Interestingly, behavioral and synaptic deficits in STOP null mice are specifically alleviated with neuroleptic treatments (Andrieux et al 2002; Brun et al 2005; Fradley et al 2005). Neuroleptics are modulators of neurotransmitter receptors, namely dopamine blockers, which have been discovered in the fifties and which are still the most active known compounds for the treatment of schizophrenia.

The possible involvement of microtubule dysfunction in schizophrenia suggests that it may be possible to amend schizophrenic disorders using microtubule drugs. Here, we have tested whether a microtubule drug could emulate neuroleptics in alleviating STOP-null mice deficits. We have used epothilone D, a taxol-related compound, which interacts directly with tubulin to stabilize microtubules (Kolman 2004; Nettles et al 2004; Wang et al 2005). In contrast with taxol, epothilone D can cross the brain blood barrier and accumulate in brain tissues. At high doses, epothilone D blocks microtubule dynamics and thereby cell division, and is currently used in clinical trials for the treatment of various tumors (Wang et al 2005). In this study we find a dramatic beneficial effect of epothilone D on STOP null mice deficits at doses which are orders of magnitude lower than anti-mitotic doses (Wang et al 2005) and which have no obvious deleterious effects on mouse fertility, embryonic development or viability. Our data provide the first demonstration that a microtubule drug can have a beneficial effect on synaptic transmission and on behavior in an animal model displaying neuronal connectivity disorders, thereby opening new possibilities for the development of novel antipsychotic agents for the treatment of human mental diseases.

Methods

Cell culture and microtubule stability assay

Hippocampal cell cultures were prepared as previously described (Dotti et al 1988). Briefly, hippocampi brain tissue from E18.5 mice were removed and digested in 0.25% trypsin in Hepes-buffered Hanks' balanced salt solution (HBSS) at 37°C for 15 minutes. After manual dissociation, cells were plated at a concentration of 5,000–15,000 cells/cm^2 on poly-L-lysine (Sigma) coated coverslips in DMEM - 10% FBS. One hour after plating the medium was changed to DMEM containing B27 and N2 supplement (Invitrogen). After one week in cultures, neuronal cells were incubated with various amount of Epothilone D for 1 hour and either exposed to cold temperature (45 minutes, 4°C) or maintained at 37°C. Cells were permeabilized in lysis buffer (30 mM Pipes, 1 mM EGTA, 1 mM $MgCl_2$, 10% glycerol, 0.1% Triton X-100, pH 6.75) for 1 minute, and processed for immunofluorescence. Cells were fixed for 6 minutes with cold methanol and incubated with mAb against α-tubulin (TUB2.1) diluted 1/100 (Sigma) for 45 minutes in PBS - Tween 0.5% and with secondary antibodies for 40 minutes. Cells were analyzed with an inverted microscope Axioscop 50 (Zeiss) controlled by Metaview (Universal Imaging, Downingtown, PA). Images were digitalized using a Coolsnap ES camera (Roper Scientific).

Drug administration

For long-term neuroleptics treatment, mice were subjected to daily administration of a combination of haloperidol and chlorpromazine (0.5 mg/day/kg and 5 mg/day/kg, respectively), for 3 months, starting at weaning, in the drinking water (Andrieux et al 2002). Epothilone D and epothilone B were provided by the GBF (Braunschweig, Germany) (Hardt et al 2001). Epothilone D was injected intra-peritoneally, once a week, at doses varying from 3 to 0.03 mg/kg/week, in 200 µl, for at least 8 weeks, in adult animals. The drug was diluted

in warm water from a 50 mg/ml stock solution in DMSO. In controls, vehicle injections consisted of 200 µl of corresponding mixtures of DMSO alone in warm water.

Synaptic function and dopamine transmission

The surface density of synaptic vesicles and synaptic plasticity were assessed in hippocampal glutamatergic neurons, as in (Andrieux et al 2002). Dopamine transmission was assessed in the nucleus accumbens as in (Brun et al 2005).

Behavioral studies

In all tests performed, STOP null mice and WT control littermate mice arose from the same colony (F1 generation from BALBc/ 129 SvPas crossing). All experiments were done blind to genotype and to treatment.

Mouse spontaneous activities (feeding, sleeping, grooming, walking or remaining still while awake) were video-recorded over a 3 h time duration during the night and the number of shifts between activities was determined (Andrieux et al 2002).

The maternal behavior of treated and untreated mice was monitored by assaying the mouse ability to build a nest and to retrieve pups. For assessment of nesting capacity, the tested mouse was placed in a 240 x 240 x 120 mm cage containing litter and provided with a Kleenex tissue folded in 4 (final dimensions, 100 x 100 mm). After 60 hours, the mouse ability to use the paper and to build a nest was scored as follows: tissue use (score as follow, 0: the Kleenex tissue remained folded; 1: the tissue has been unfolded but not shredded; 2: the tissue was shredded), nest building (score as follow, 0: no attempt to build a nest; 1: primitive flat nest of uncontrolled shape; 2: true nest, the paper is mixed with litter to form a circular nest, less than 80 mm in diameter). For assessment of retrieving ability, following the nest-building test, on day one, each female was exposed to three 1-3-day-old pups. Pups were placed in three corners of the cage distant from the nest. After 30 min the pups were returned to their natural mother. On day two, each female was again exposed to three pups. The

number of pups retrieved during 30 min by each female was scored. Retrieving was scored as the number of pups retrieved (0 to 3). Finally, a global score for nesting and retrieving capacity (TNR) was determined for each mouse, by adding the tissue use, nest building, and pup retrieving scores (maximal score for TNR is 7).

Results

Microtubule stabilization by epothilone D in STOP null neurons

Neuronal microtubules are normally cold stable, due to association with STOP proteins (Guillaud et al 1998) (Figure 1A-B). Microtubule cold stability is thus abolished in STOP deficient neurons (Andrieux et al 2002) (Figure 1C-D). To test the microtubule stabilizing effect of epothilone D, STOP deficient neurons were exposed to various drug concentrations and then either kept at 37°C or exposed to the cold for 45 minutes prior to microtubule staining. At subnanomolar concentration of epothilone D, cold exposure induced extensive microtubule disassembly (Figure 1D-E). At 1nM concentration epothilone D induced an apparently complete cold-stabilization of neuronal microtubules (Figure 1F), with no more detectable difference in microtubule signals between cold exposed STOP null neurons and control cells and no further increase in the microtubule signal at higher epothilone D concentrations (Figure 1G-H).

In whole animals, in this study, epothilone D was generally used at a dose of 3 mg/kg/week, in one intra-peritoneal injection per week. This drug dosage is ten times lower than the dosage routinely used for inhibition of tumor growth in mice (Wang et al 2005), and has been found to yield a brain epothilone concentration in the nanomolar range (Schering AG patent, WO 03/074053 A1, published 12.09.2003).

Synaptic vesicle density

A striking signature of STOP deficiency is a two fold drop in the average surface density of synaptic vesicle on electron micrographs of hippocampal glutamatergic synapses (Andrieux et al 2002). In addition to its physiological importance, synaptic vesicle density shows little variability between animals of the same genotype and between repeated experiments, and offers thereby a meaningful and sensitive way to detect treatment effects. In this study, epothilone D treatment had no significant effect on the vesicle density in WT mice (Figure 2), but, in contrast, induced a 16%, highly significant increase of the vesicle density in STOP null mice (Figure 2). In parallel experiments, a long-term neuroleptic treatment (Andrieux et al 2002) induced a 19% increase in the vesicle density, in STOP null mice (Figure 2).

Synaptic plasticity

STOP null mice display defects in both short- and long-term synaptic plasticity, including impaired Post-Tetanic Potentiation (PTP), Long-Term Depression (LTD) and Long-Term Potentiation (LTP) in the CA1 region of the hippocampus (Andrieux et al 2002). Neuroleptic treatment restores PTP in STOP null mice, in the absence of significant influence on LTP (Andrieux et al 2002). In the present study, epothilone D treatment improved significantly both PTP and LTP in STOP null mice (Figure 3A-B). In LTP experiments, the restoration of synaptic potentiation by epothilone D treatment was evident at 60 min and persisted at subsequent time points (up to 120 min, Figure 3B). In contrast, no significant effect of the treatment was observed for LTD (Figure 3C). In WT animals, synaptic plasticity was not significantly affected by epothilone D treatment (Figure 3, A-C). Thus, epothilone D treatment improves both PTP and LTP in STOP null mice, and does not detectably affect synaptic plasticity in WT animals.

Dopamine transmission

In STOP null mice, dopaminergic neurons in the nucleus accumbens (Nacc) exhibit a dopaminergic hyper-reactivity, with a conspicuous augmentation of dopamine efflux evoked by electrical stimulations, compared to WT neurons (Brun et al 2005). In the present study, dopamine effluxes were abnormally high in WT animals, most probably due to the stress generated by weekly injections (Pani et al 2000). In our experimental conditions, the dopamine response was unaffected by epothilone D treatment in both WT and STOP null mice (Figure 4).

Behavioral analysis

STOP null mice display a complex set of behavioral disorders several of which, such as anxiety or locomotor activity, proved hard to test on animals stressed by weekly injections. In this study, we used both an analysis of spontaneous activity, and an analysis of maternal behavior, which was not detectably perturbed by injections.

Mouse spontaneous activity is disorganized in STOP null mice, with an increased frequency of shifts between different spontaneous activities, such as feeding, walking, remaining still without sleeping, sleeping and grooming (Andrieux et al 2002). Epothilone D treatment induced a significant decrease in the frequency of shifts between activities in STOP null mice, in the absence of significant effects in WT animals (Figure 5A).

STOP null mice display severe maternal defects, which are of behavioral origin, which affect tasks such as nest building or pup retrieving, and which we have previously used as end-points for assessment of drug effects (Andrieux et al 2002). When given paper tissue, WT nulliparous females first shred the tissue and then use the shredded tissue to build a nest. When properly trained and presented with pups, such mice are also capable to retrieve pups. We scored mouse ability to process tissue (T), to use the tissue to build a nest (N) and to retrieve pups (R). The three scores were added to determine a global maternal ability score

(TNR). WT mice had, as expected, good maternal skills in these tests, and their performances were unaltered by epothilone D treatment (Figure 5B). In contrast, vehicle alone treated STOP null mice were conspicuously incompetent in maternal tasks (Figure 5B). The standard treatment with 3 mg/kg/week induced a remarkable improvement of maternal behavior in STOP null mice, with a highly significant increase in the global maternal ability score (Figure 5B). Epothilone D was still fully efficient at a 0.3 mg/kg/week, whereas drug effects were barely detectable at 0.03 mg/kg/week.

Nurturing deficits invariably lead to pup death in the offspring of STOP null mice (Andrieux et al 2002). In a previous study, long-term neuroleptic treatment improved the maternal skills of some STOP null mothers to an extent sufficient to allow pup survival (Andrieux et al 2002). Here, in 17 vehicle alone treated STOP-null females, no pup survival was observed, in agreement with previous observations made on dozens of STOP null females and hundreds of pups (Andrieux et al 2002). In contrast, pup survival was observed in the offspring of 5 out of 15 STOP null mice treated with epothilone D. In two cases, the amelioration of maternal skills was truly remarkable, with a survival of all the pups from the litter (7/7 and 6/6).

Drug tolerance

Although a systematic study of drug tolerance would be beyond the scope of our work, we have checked for possible obvious side effects of epothilone D in WT animals. The longest followed animals were three females, injected with 3 mg/kg/week for 15 months. These animals showed no obvious behavioral or anatomical anomalies. A group of 48 females treated with 3 mg/kg/week of epothilone D followed for 4-8 months showed no obvious anomalies. To test for a possible deleterious effect of epothilone D on female fertility and development, we monitored WT females exposed either to 0.3 mg/kg/week epothilone D or to

vehicle alone, for a 5 month time period during which they were mated and underwent pregnancy (n=7, in each group). All females gave birth to apparently normal pups, which later developed normally. The number of pups per mother in the epothilone treated group (5.6 ± 0.72) was similar to that observed in the vehicle alone injected group (5.00 ± 1.03). These results indicate an absence of obvious deleterious effect of epothilone D treatment on mouse embryonic development, viability or fertility, at the doses used in this study.

Discussion

Microtubules are major components of neurons. Since synaptic plasticity depends on microtubule functions (Dillon and Goda 2005; Okamoto et al 2004; Zito et al 2004) a role of microtubules in synaptic transmission and hence in mental functions has long been suspected (van Rossum and Hanisch 1999), but not firmly demonstrated. However, convergent evidence for a role of the cytoskeleton in mental illnesses has arisen recently, with the discovery of a cytoskeletal role for DISC1, a schizophrenia susceptibility gene (Callicott et al 2005; Morris et al 2003; Owen et al 2005a) and of a function in the synapse organization for other genes involved in schizophrenia (Harrison and Owen 2003; Harrison and Weinberger 2005; Owen et al 2005a; Owen et al 2005b; Petryshen et al 2005; Stefansson et al 2004) or in other severe mental pathologies (Chubykin et al 2005; Jamain et al 2003; Laumonnier et al 2004; Yan et al 2005). Our work with STOP null mice (Andrieux et al 2002) has demonstrated that alterations of a microtubule effector could generate a “disease of the synapse” (Mirnics et al 2001) with a set of neurotransmission defects considered as central to mental diseases such as schizophrenia (Frankle et al 2003), associated with schizophrenic-related behavioral disorders. Finally, very recently, the deletion of the cytoskeletal protein Stathmin has been shown to induce behavioral disorder in mice (Shumyatsky et al 2005). Our present

demonstration that a microtubule stabilizer can amend synaptic defects and can improve dramatically behavior in STOP null mice, adds strong evidence for an important role of microtubules in synaptic modulation and in mental functions.

The positive influence of epothilone D on synaptic plasticity and behavior raises many challenging questions. The most obvious ones concern the relationship between microtubule stabilization and the drug effects. In the present study, we find that nanomolar concentrations of epothilone D are sufficient to rescue microtubule cold-stability in STOP deficient neurons, *in vitro*. Nanomolar concentrations of epothilones have previously been shown to stabilize microtubule dynamics in different cell types, presumably due to strong accumulation of the drugs inside cells (Chou et al 1998; Hofle and Reichenbach 2005). Such nanomolar epothilone concentrations also correspond to the range of brain drug concentrations observed in mice, in treatment conditions close to ours (3mg/kg) (Schering AG patent, WO 03/074053 A1, published 12.09.2003). Thus, with rough estimates, there is no major incompatibility between *in vitro* and *in vivo* data with regard to epothilone effects. Progress in microscopy technology may allow in a foreseeable future, direct studies of microtubule dynamics during synaptic activation, and of the influence of microtubule dynamics on the size of vesicle pools and on synaptic plasticity, both in normal conditions and in the presence of epothilone D.

In STOP null mice, the effects of epothilone D on behavior are reminiscent of those of a chronic neuroleptic treatment. Additionally, in agreement with previous studies (Konradi and Heckers 2001), we find that neuroleptics are able to rescue synaptic plasticity (Andrieux et al 2002) and synaptic vesicle pools (this study) in glutamatergic neurons, as epothilone D. However, whereas neuroleptics are thought to act principally as dopamine blockers, epothilone D had no detectable effect on dopamine transmission, in our treatment conditions. Thus, a long-term epothilone D or neuroleptic treatments may act through distinct pathways

and an association of neuroleptic and epothilone D may turn out to be more efficient than each drug used alone.

The lack of effect of epothilone D on dopaminergic efflux observed in this study whereas the drug apparently ameliorates glutamatergic transmission may mean that hyper-dopaminergy is not a direct result of hypo-glutamatergy in STOP null mice. However, such a conclusion could be premature, since, in our study context, dopamine transmission is most probably affected by the chronic stress induced by the repeated intra-peritoneal injections used for long-term epothilone treatment (Pani et al 2000; Thompson et al 2004).

We have chosen epothilone D among available epothilones because it has less toxicity at efficient doses than other epothilones and because it accumulates in brain tissues upon chronic administration (Hofle and Reichenbach 2005). In our experience, epothilone D could be used at low doses provided that treatment duration was sufficient and we believe that this was a key factor for success. We have done some attempts with epothilone B, but in this case, there was an apparent overlap between toxic doses and effective doses. Although there is little left unknown about the molecular targets of epothilones whose complex with tubulin has been crystallized (Nettles et al 2004) it will obviously be interesting to find compounds other than epothilone D with similar activity on synaptic transmission and on behavior. Epothilone research is currently very active, and new compounds with similar pharmacological profile as epothilone D will undoubtedly be available in the near future.

Is the beneficial effect of epothilone D on behavioral disorders special to STOP null mice, which are deficient for a microtubule effector, or will they be observed in other animal models of mental diseases? Tests of epothilone D in different animal models will tell whether modifying microtubule dynamics can have a favorable effect even in models not directly involving cytoskeletal molecules. With respect to human schizophrenia, at least a subclass of

cases may involve the cytoskeleton directly (Owen et al 2005a) or indirectly, and may thereby benefit from epothilone D treatment.

In conclusion, the beneficial effect of epothilone D treatment on synaptic transmission and behavior observed in an animal model of schizophrenia suggests that microtubules or microtubule effectors represent useful novel targets for anti-psychotic agents. Epothilone D is already validated for human use and data should be available concerning its possible side effects in humans at the low doses required for psychotropic effects. Therefore, testing epothilone D in schizophrenic patients or in patients with other mental diseases thought to result from neuronal connectivity defects may turn out to be feasible in the near future.

Acknowledgments: We thank D. Proietto for technical help, Dr J.M. Danion for helpful discussion, Dr C. Bosc for discussion and support.

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Figure legends

Figure 1: Microtubule stability

Analysis of microtubule cold stability in primary cultures of neuronal cells from wild type (A-B) and STOP deficient embryos (C-H) in the presence or not of various amount of Epothilone D. Cells were either maintained at 37°C or exposed to the cold (0°C for 45 min). Following free tubulin extraction by cell permeabilization, microtubules were stained with anti α-tubulin (green) and nuclei were stained with Hoescht 33258 (blue).

Figure 2: Analysis of the surface density of synaptic vesicles

Synaptic vesicle density, calculated as the ratio of the number of vesicles/nerve terminal surface (after subtraction of the surface area occupied by mitochondria). Results are shown as means +/- s.e.m. Treatments and genotypes were as indicated. In epothilone experiments, 75 measurements from five mice, in each treatment and genotype group were performed. In neuroleptic experiments, 150 measurements from three animals were performed in each treatment group. *** $p< 0.001$, t-test.

Figure 3: Quantitative analysis of synaptic plasticity in CA1 region of hippocampus

Averaged responses are shown in the two left panels and computed results are illustrated in the right panels. Genotypes and treatments, as indicated.

A. PTP (Post-tetanic potentiation) of Schaffer collateral synaptic transmission. At time 0 a high-frequency stimulation (100 Hz, 1 sec) of Schaffer collaterals was applied in the presence of the NMDA antagonist D-APV (50-100 µM). Left panels: summary of PTP experiments in Epothilone D treated and untreated WT and STOP null mice. Right panel: average responses

0-30 sec after tetanus, showing no modification of potentiation in treated WT mice (n = 7) versus untreated WT mice (n = 8) whereas a significant increase of potentiation was observed in treated STOP null mice (n = 7) versus untreated STOP null mice (n = 8).

B: LTP experiments at the Schaffer collateral-CA1 pyramidal cell synapses. A high-frequency stimulation (four tetani of 100 Hz, duration 1 sec stimuli, given 20 sec apart) was done at time 0. Initial EPSP slopes were normalized in each experiment using the averaged slope value during the control period (-10 to 0 min). Left panels: summary of LTP experiments (mean ± s.e.m.) done in epothilone D treated and untreated WT or STOP null mice. Right panel: results computed at 50-60 min after tetanus showed no significant modifications of potentiation in treated WT mice (n = 9) compared to untreated mice (n = 6) whereas a significant increase of the potentiation was observed in treated STOP null mice (n = 8) compare to untreated mice (n = 9). Results computed at 110-120 min also showed a significant increase of the potentiation in treated STOP null mice (n = 8) compare to untreated mice (n = 8).

C: LTD experiments at the Schaffer collateral-CA1 pyramidal cell synapses. At time 0, a low-frequency stimulation (1 Hz, 15 min) of Schaffer collaterals was applied. Right panels, summary of LTD experiments in epothilone D treated and untreated WT or STOP null mice. Right panel, results computed at 50-60 min after stimulus showed no significant modifications of LTD in WT treated mice (n=6) versus untreated mice (n=8) or in treated STOP null mice (n=5) versus untreated mice (n= 6).

* $p<0.05$, ** $p<0.02$, t test.

Figure 4: Effect of epothilone D on evoked dopamine efflux

DA effluxes in response to electrical stimulations were measured by differential pulse amperometry in the nucleus accumbens of WT or STOP null mice (STOP KO), treated either

with vehicle alone or with epothilone D. Electrical stimulations, applied in the ascending DA pathway, consisted of 200 pulses with a regular pattern, at various frequencies as indicated. The effect of stimulation frequency is presented as mean absolute values ± s.e.m. of the maximal evoked DA efflux changes (n = 8-10 in each group). The DA response was not affected by epothilone D in either WT or STOP null mice ($p>0.05$, at all time points, t test).

Figure 5: Behavioral studies

A: Mouse activities. Spontaneous mice activities (sleeping, feeding, grooming, walking and remaining while awake) were video-recorded during 3 h, in WT or STOP null mice (STOP KO) treated either with epothilone D (Epo D 3mg/kg/week) or with vehicle alone. The number of occurrences of each activity was determined for each mouse and averaged: WT vehicle n = 29 (12 males and 17 females), WT Epo D n = 33 (12 males and 21 females), STOP KO vehicle n = 28 (12 males and 16 females), STOP KO Epo D n = 33 (12 males and 21 females). Each block corresponds to an activity as indicated. Results were similar in males and in females and were pooled for analysis.

B: Maternal behavior. The nurturing ability of WT and STOP null mice (STOP KO) either treated with Epothilone D, at different concentrations or with vehicle alone was assessed (see material and methods). Score ranges were 0-2 for tissue use (T), 0-2 for nest building (N), 0-3 for the number of retrieved pups (R), and 0-7 for the global nurturing score (TNR). TNR were calculated for each mouse and averaged (n = 17 for all groups). Each block represents a maternal skill, as indicated.

* $p<0.05$, ** $p< 0.01$, *** $p<0.001$, non parametric Mann and Whitney U test

Figure 1

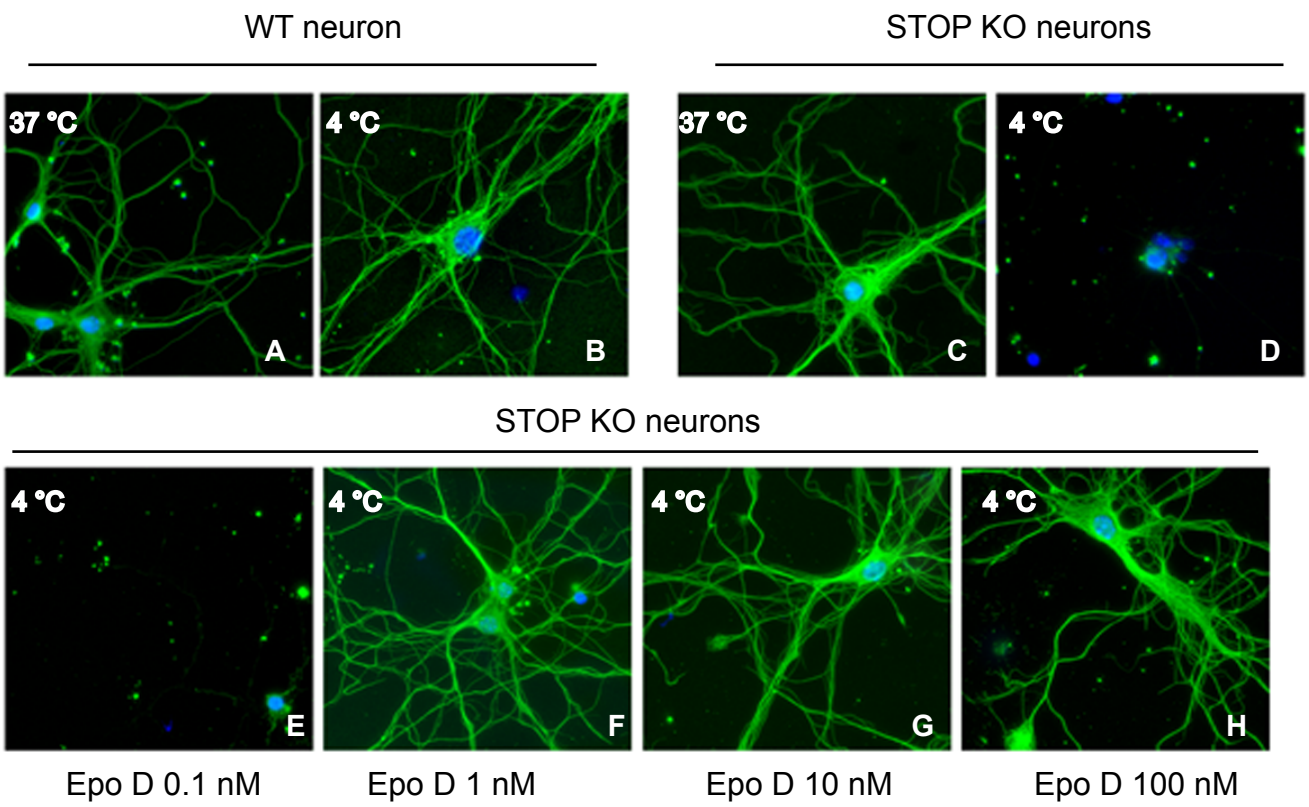

WT neuron
STOP KO neurons
37 °C
A
4 °C
B
37 °C
C
4 °C
D
STOP KO neurons
4 °C
E
4 °C
F
4 °C
G
4 °C
H
Epo D 0.1 nM
Epo D 1 nM
Epo D 10 nM
Epo D 100 nM

Figure 2

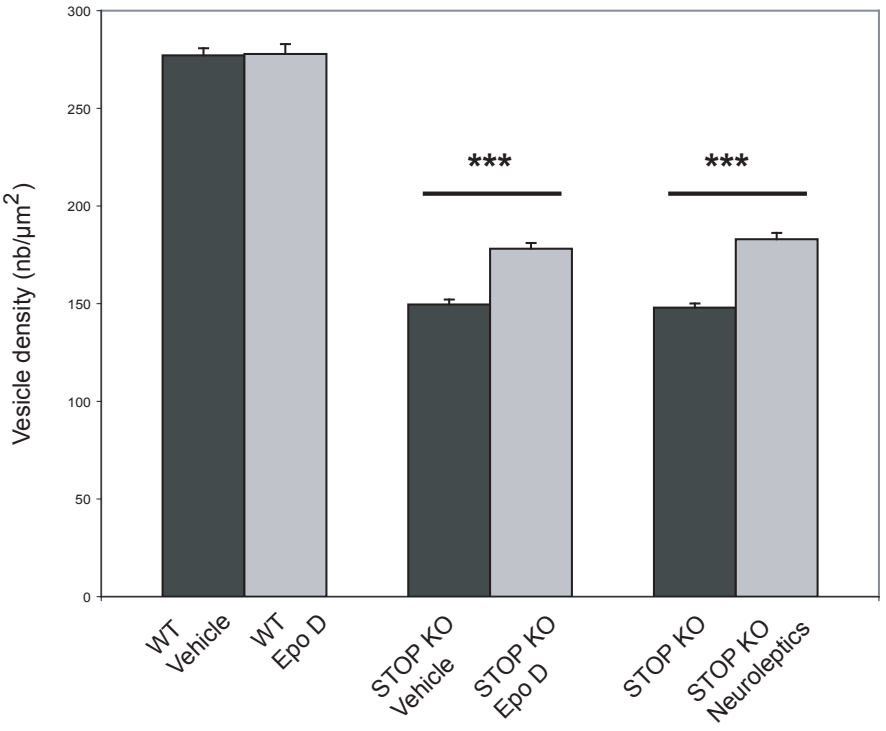

300
250
200
150
100
50
0
Vesicle density (nb/µm2)
***
***
WT Vehicle
WT Epo D
STOP KO Vehicle
STOP KO Epo D
STOP KO
STOP KO Neuroleptics

Figure 3

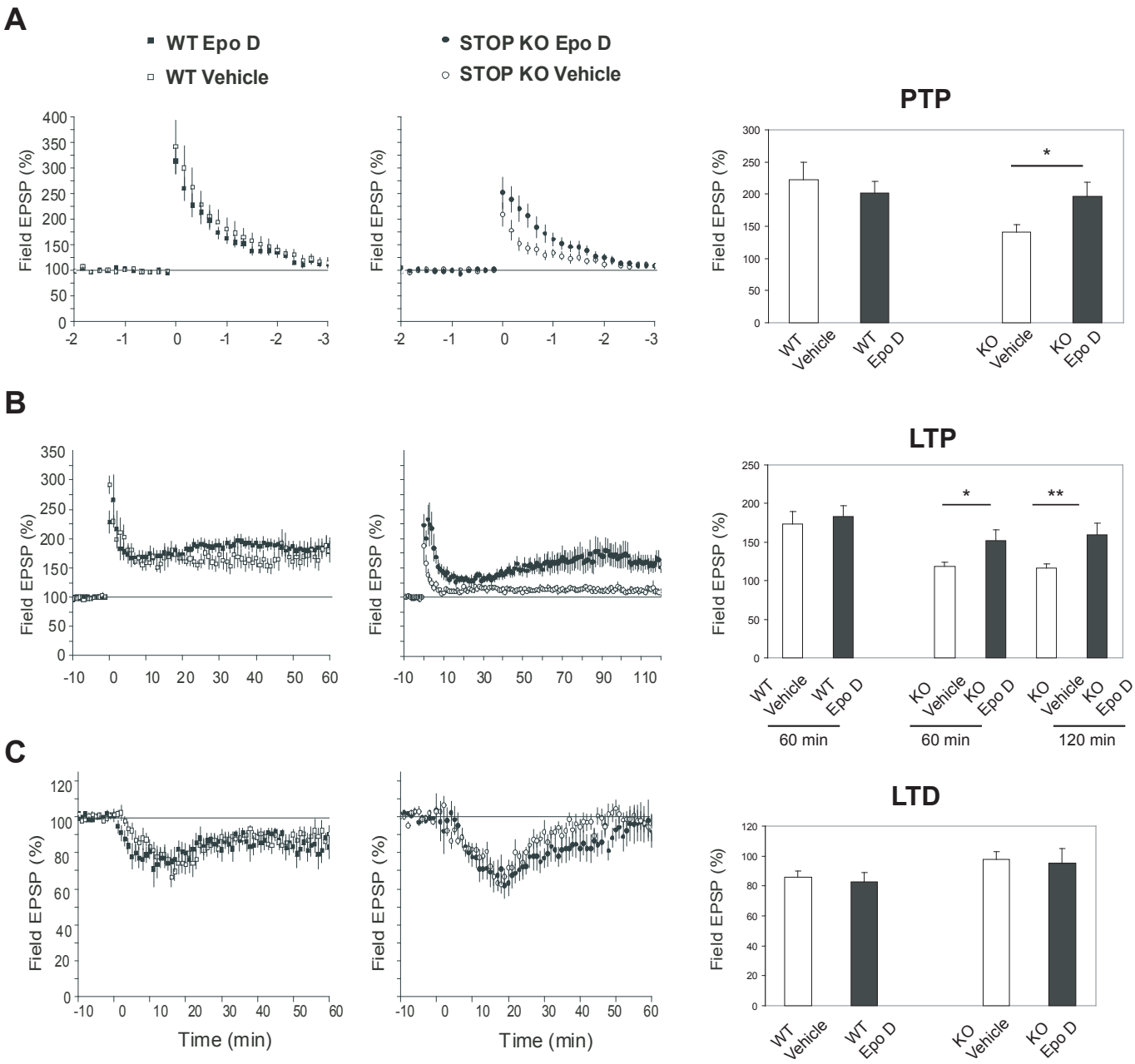

A
WT Epo D
WT Vehicle
STOP KO Epo D
STOP KO Vehicle
Field EPSP (%)
PTP
WT Vehicle
WT Epo D
KO Vehicle
KO Epo D
B
LTP
60 min
60 min
120 min
C
LTD
Time (min)
Time (min)

Figure 4

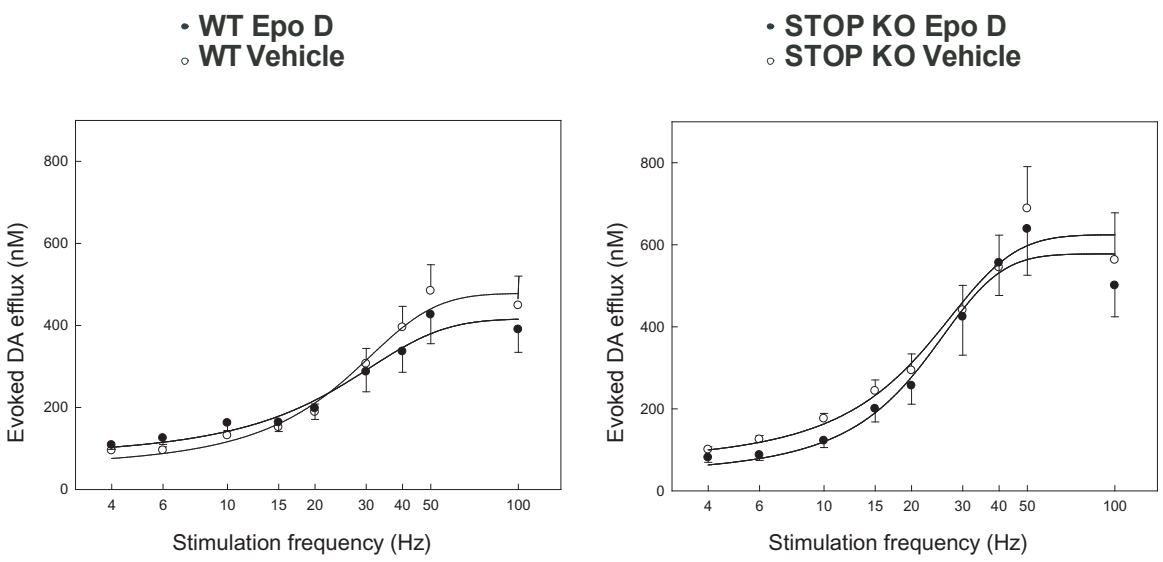
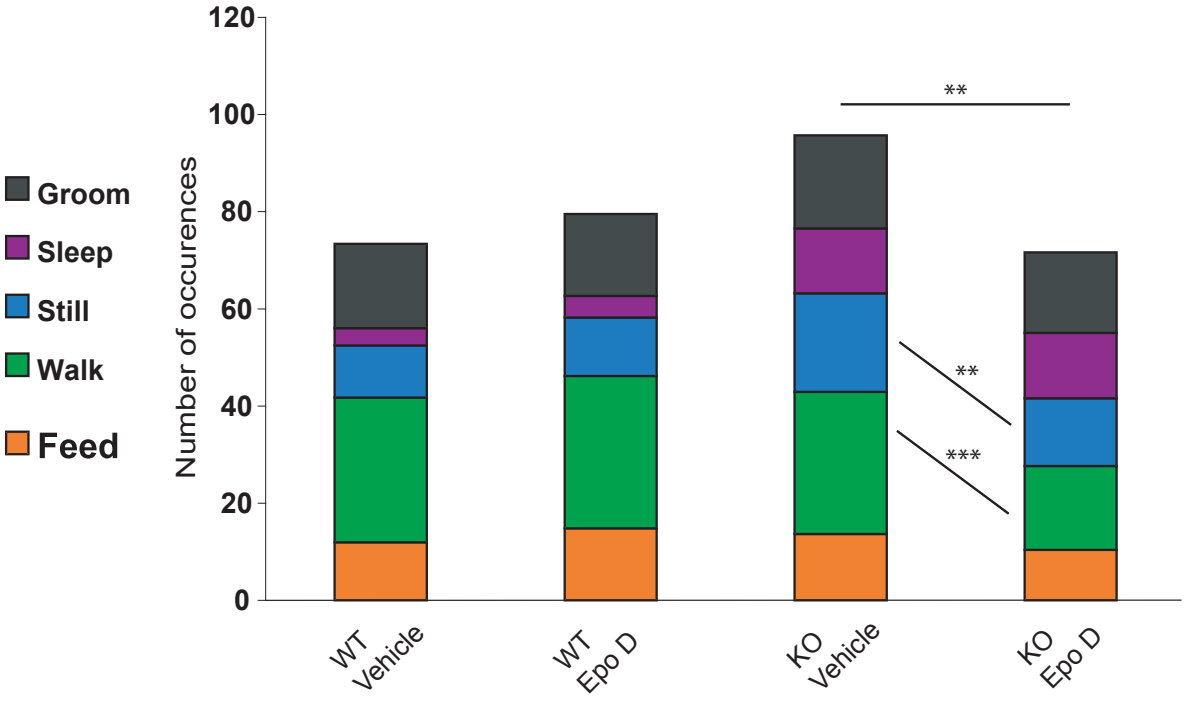

• WT Epo D
○ WT Vehicle
• STOP KO Epo D
○ STOP KO Vehicle
Evoked DA efflux (nM)
0
200
400
600
800
4
6
10
15
20
30
40
50
100
Stimulation frequency (Hz)

Figure 5

A Mouse activities



B Maternal behavior

