# Role of topiramate on impulsive behavior in DBA/2 mice

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## MATERIAL AND METHODS

### *Animals*

Male AJOlaHsd and DBA/2OlaHsd mice (age 8-10 weeks, 20-25 g) were purchased from Harlan (Barcelona, Spain). Mice were housed under controlled conditions (23 ± 2ºC; 12h standard light/dark cycle). Standard laboratory chow and water were available ad libitum in all procedures except in the delayed reinforcement task in which 1 hour/day chow access was allowed to increase task motivation. All experiments were in accordance with guidelines established by the European Council Directive (86/609/EEC) and were approved by the Institutional Animal Care Committee.

### *Drugs*

Cocaine clorhidrate (20 mg/Kg - Spanish Ministry of Health) was administered i.p. in the conditioned place preference paradigm immediately before placing mice in the apparatus. Topiramate/TPM (12.5, 25 and 50 mg/Kg - Topamax® by Janssen-Cilag) was administered p.o. in the delayed reinforcement task 1 hour prior testing following two administration patterns. In the acute pattern mice received TPM p.o. once a day 1 hour prior testing during 10 days (delay phase) and in the chronic pattern there were a pre-treatment (7 days) and a treatment during all the task (training + delay phase) administering TPM p.o. twice a day every 12 h.

### *Behavioral paradigms*

#### -*Delayed reinforcement task*

The evaluation of delay discounting and behavioral inhibition was carried out in operant chambers which consist of a 20 x 20 x 25 cm metal/acrylic square box equipped with two levers, one feeder device with a magazine to drop food pellets, one stimulus light and one stimulus buzzer (Panlab, Barcelona, Spain). These operant boxes are isolated inside soundproof chambers equipped with a chamber light and a fan. In the training phase, one lever press delivered one food pellet (immediate lever - IL), whereas the other lever activated stimulus light and buzzer immediately delivering three food pellets (delayed lever - DL). A 30-s time-out was established during which additional lever-presses in either lever were recorded but without consequence. When animals achieved acquisition criteria (>75% preference for delayed lever, at least 10 reinforced trials per session and <20% of lever presses deviation for 3 days) they were moved to the test phase (10 days), a time delay was introduced between lever pressing in the delayed lever and the delivery of the three pellets. Delay was increased day by day in a between session way (0, 6, 12, 18, 24, 30, 42, 54, 66, 78, 90 s). Cognitive (change of % preference for DL) and motor (number of IL presses during delay) impulsivity was assessed in this task.

#### -*Conditioned place preference*

The conditioned place preference (CPP) apparatus consisted in two chambers of 30 x 20 x 20 cm separated by a sliding door. The conditioning chambers were black with a stainless steel grid floor and colored with a smooth plasticized floor. The CPP procedure (1) consisted of three phases as follow: A) Preconditioning phase: Day 0, mice were placed between the two chambers to allow free exploration of both chambers for a period of 15 min. Mice spending more than 70% of time in any of two chambers were discarded from the experiment. B) Conditioning phase: Half of the animals of each genotype received saline (i.p.) in the colored chamber while the other half in the black chamber on days 1, 3, 5, 7 and 9. The same animals received cocaine (20 mg/kg; i.p.) in the opposite chamber on days 2, 4, 6, 8, and 10. Animals remained in the conditioned chamber during 20 min. C) Test time (day 11): mice did not received cocaine or saline, the door was opened and mice were placed in the middle of two chambers to allow free exploration of two compartments. The time that mice spend in each chamber was recorded during 15 min, evaluating the % of preference for the drug-paired side. After the test phase on day 11, animals conditioned with cocaine underwent an extinction session schedule which consisted of the placement of the animals in the apparatus for 15 min. No drugs were administered in this sessions. The criterion to consider the preference extinguished was the lack of statistical significance between the time spent in the drug-paired and in the saline-paired compartments. The day after the extinction, reinstatement was evaluated administering a priming cocaine dose (20 mg/Kg; i.p.) and allowing animals free ambulation for 15 min between compartments.

### *Gene expression analyses*

#### -*Real time-qPCR*

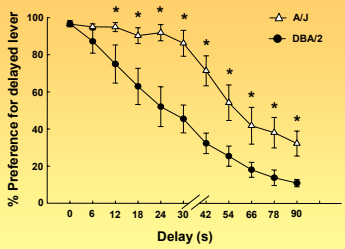
Mice were sacrificed and brains were removed from the skull and frozen over dry ice. Coronal brain sections (500 µm) were obtained according to Paxinos and Franklin (2) in a cryostat (-10ºC) and the prefrontal cortex (PFC) and nucleus accumbens (ACC) were microdissected according to Palkovits method (3). Total RNA was isolated from brain tissue micropunches using TRI reagent (Applied Biosystems, Foster City, CA) and subsequently retrotranscribed to cDNA. Quantitative analysis of the relative abundance of dopamine 2 receptor ($DrD_2$) gene expression was performed on the ABI PRISM 7500 Sequence Detector System (Applied Biosystems, Foster City, CA). The reference gene used was 18S rRNA, detected using Taqman ribosomal RNA control reagents. Briefly, data for each target gene were normalized to the endogenous reference gene, and the fold change in target gene mRNA abundance was determined using the $2^{-\Delta\Delta Ct}$ method (4) so that DBA/2 or DBA/2 TPM-treated mice levels were expressed relative to A/J or DBA/2-control mice levels, respectively.

### *Statistical analyses*

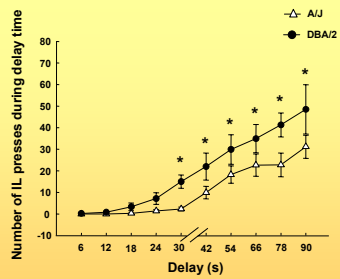
Statistical analyses were performed using Student t-test, one or two-way analysis of variance followed by the Student Newman Keul's test. Differences were considered significant if the probability of error was less than 5%. SigmaStat v3.11 software was used for all statistical analyses.

## DIFFERENT IMPULSIVITY LEVEL BETWEEN A/J AND DBA/2 STRAINS OF MICE

### DELAYED REINFORCEMENT TASK



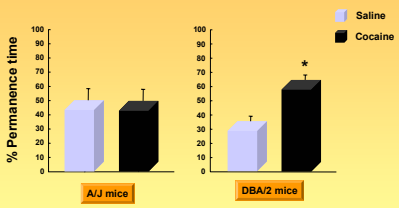

Cognitive impulsivity level was investigated with the delayed reinforcement task where delay discounting was determined between A/J and DBA/2 mice. Dots represent the means and vertical lines ± SEM of % preference for delayed reinforcement; *, values from DBA/2 mice that are significantly different from A/J mice (p<0.05).



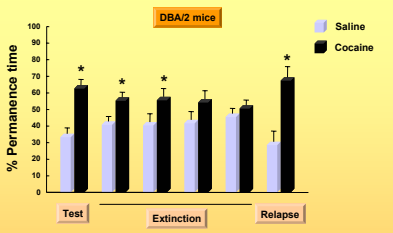

Motor impulsivity level was investigated with the delayed reinforcement task where number of lever presses during delay time were compared between A/J and DBA/2 mice. Dots represent the means and vertical lines ± SEM of number of lever presses during the different delay times; *, values from DBA/2 mice that are significantly different from A/J mice (p<0.05).

## DIFFERENCES IN PLACE CONDITIONING DEPENDING ON IMPULSIVITY LEVEL

### CONDITIONED PLACE PREFERENCE



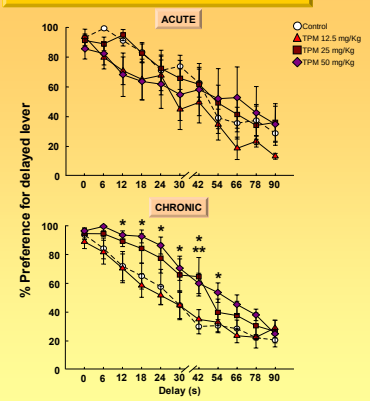

Place preference evaluation in the test phase of the conditioned place preference paradigm between A/J and DBA/2 mice. Columns represent the means and vertical lines ± SEM of % permanence time in saline- or cocaine-paired sides; *, cocaine-paired side % permanence time values that are significantly different from saline-paired side (p<0.05).



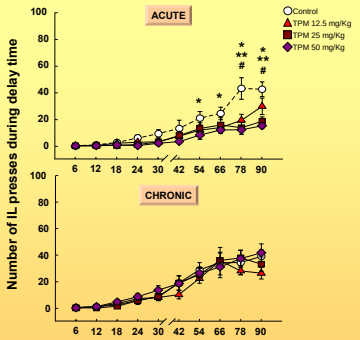

DBA/2 mice preference for cocaine-paired side was extinguished in the conditioned place preference apparatus (free exploration). Relapse was achieved with a cocaine priming dose (20 mg/Kg). Columns represent the means and vertical lines ± SEM of % permanence time in saline- or cocaine-paired sides; *, cocaine-paired side % permanence time values that are significantly different from saline-paired side (p<0.05).

## EFFECTS OF TOPIRAMATE ON DBA/2 MICE COGNITIVE AND MOTOR IMPULSIVITY LEVEL

### DELAYED REINFORCEMENT TASK



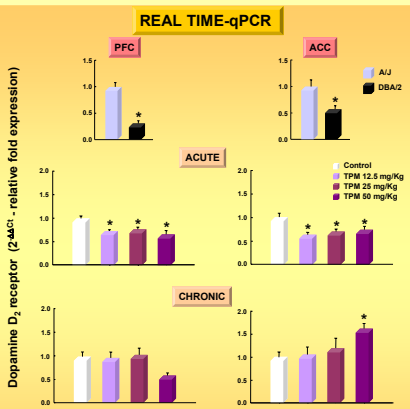

Cognitive impulsivity level was investigated with the delayed reinforcement task. Delay discounting was determined between treatment groups. Dots represent the means and vertical lines ± SEM of % preference for delayed reinforcement; *, values from TPM 50 mg/Kg group that are significantly different from control mice (p<0.05); **, values from TPM 25 mg/Kg group that are significantly different from control mice (p<0.05).




Motor impulsivity level was investigated with the delayed reinforcement task. Number of IL presses during delay time were compared between treatment groups. Dots represent the means and vertical lines ± SEM of number of IL presses during the different delay times; *, values from TPM 50 mg/Kg groups that are significantly different from control mice (p<0.05); **, values from TPM 25 mg/Kg groups that are significantly different from control mice (p<0.05); #, values from TPM 12.5 mg/Kg group that are significantly different from control mice (p<0.05).

## GENE EXPRESSION ANALYSES

### REAL TIME-qPCR




Dopamine $D_2$ receptor gene expression in the PFC and ACC. Gene expression in DBA/2 mice is expressed relative to A/J mice, and gene expression in DBA/2 TPM-treated mice is expressed relative to DBA/2 control mice. Columns represent the means and vertical lines ± SEM of $2^{-\Delta\Delta Ct}$; *, values from DBA/2 or DBA/2 TPM-treated mice that are significantly different from A/J or DBA/2 control mice (p<0.05), respectively.

**REFERENCES**

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## CONCLUSIONS

- A/J and DBA/2 inbred strains of mice have an opposite impulsivity level that influences cocaine conditioned place preference. A high basal impulsivity level predisposes to a higher attentional bias.
- Topiramate administration is able to modulate motor impulsivity acutely and cognitive impulsivity chronically in high-impulsive DBA/2 mice.
- DBA/2 mice have a lower level of dopamine $D_2$ receptor gene expression than A/J mice in PFC and ACC. Topiramate reduced acutely dopamine D2 receptor gene expression in PFC and ACC, but chronically did not have effect in PFC and produced an increase in ACC.
- These findings show for the first time that the therapeutic usefulness of topiramate in drug dependence could lie in its anti-impulsive effects, which depend on the administration pattern. In addition, these effects might be related with the regulation of cortico-striatal dopamine.

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