

Supplementary Information for

Profound alteration in reward processing due to a human polymorphism in *CHRNA5*: a role in alcohol dependence and feeding behavior

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SUPPLEMENTARY METHODS

Animals

WT and α 5SNP rat lines were originally derived from the same breeders, bred and housed at the Institut Pasteur, Paris, France. Rats were 7-10 weeks at the beginning of the experiments and individually housed in a temperature-controlled environment on a 12h reverse light/dark cycle (lights off from 08:00 to 20:00). All rats received at least two weeks of acclimatization and one week of handling before experimentation.

Intermittent ethanol two-bottle choice paradigm

Rats were given one bottle with 20% EtOH (v/v) (Sigma Aldrich, Saint Quentin Fallavier, France) and one bottle with tap water in their home cage on Mondays, Wednesdays and Fridays and two bottles of tap water on Tuesdays, Thursdays and over the week-ends. Animals were given 24h access to the solutions. Food was available *ad libitum* and animal's weight was measured daily. There was no difference in weights between genotypes until the end of the experiments (Fig. S2a).

Solutions were presented in 500 ml bottles and the position of each bottle was randomly assigned in each group. Two "leak" bottles, one for water and one for EtOH (or two bottles of water during the water-only days) were used to control for accidental sipper leakage and were placed in an identical plastic cage with a wire top. Each bottle was weighed before presentation to the animal and after 24h of drinking. The amount consumed from each bottle was calculated by subtracting the amount removed from the corresponding leak bottle from the volume removed from each bottle by the animal's consumption. The % of preference for the EtOH solution was calculated with the following formula: (grams of EtOH solution consumed / (grams of EtOH solution consumed + grams of water consumed)) * 100. The amount of EtOH consumed in g/kg was calculated with this formula (((grams of EtOH solution consumed) * EtOH concentration) * 0.789) / body weight (kg).

Acquisition

The acquisition and maintenance phase consisted of 23 sessions with one bottle of EtOH and one bottle of water and 14 sessions with two bottles of water. The % of preference for EtOH and the amount of EtOH consumed in g/kg was measured for each session.

Quinine adulteration of EtOH intake

The quinine adulteration phase was conducted to test aversion-resistant alcohol intake, a procedure proposed to model pathological consumption of alcohol [1]. The timing was chosen to have optimal conditions to observe possible stronger/earlier resistance to quinine in transgenic rats, adapted from [1]. Different doses of quinine (0, 0.05, 0.1 and 0.2 g/L) (quinine hydrochloride, Sigma-Aldrich, Saint Quentin Fallavier, France) were added to the EtOH solution on the Friday sessions according to a latin-square design, except for two α 5SNP rats which showed signs of sickness. The % of preference for EtOH and the amount of EtOH consumed in g/kg was measured for each session.

Abstinence and re-exposure to EtOH

8 WT and 6 α 5SNP rats were submitted to the four-month withdrawal phase with access to two bottles of water and were re-exposed to one bottle of 20% EtOH and one bottle of water for two sessions

(with two sessions with water only between each EtOH session). The % of preference for EtOH and the amount of EtOH consumed in g/kg was measured for each session.

Statistical analysis

For two-group comparisons, data were analyzed with unpaired Student's *t* tests or Mann-Whitney U tests when normality and variance homogeneity conditions were not met for parametric test use. Two-way repeated measures ANOVAs were used (group as between- and session/dose as within-subject factors). Significant main effects ($p < 0.05$) were further analyzed using Bonferroni for multiple comparisons post-hoc tests.

Ethanol operant oral self-administration procedure

Sessions were run every day from Monday to Friday and lasted 1h unless specified otherwise. Rats were fed 20g of laboratory standard chow per day, for the whole experiment, at the end of the day to favor comparable motivational states between animals at the time of the experimental testing (from 9 am to 4 pm) (except for weekends where animals were fed on Friday evening with adapted food quantities). Such amount was enough to maintain 90% of animal free-feeding weight, thereby representing a mild food restriction that was shown to enhance motivational effects of drugs of abuse [2]. The amount of EtOH consumed in g/kg was calculated with this formula: ((volume of EtOH solution consumed (mL) / (body weight (kg))) * EtOH concentration) * 0.789. Head entries in the magazine where EtOH was delivered were not recorded but the EtOH receptacle was checked at the end of all sessions for each rat to verify EtOH consumption and to measure left ethanol if necessary (which was then taken into consideration for the calculation of EtOH intake).

Active levers (AL) and inactive levers (IL) were equally distributed between groups. Rats from each genotype were equally distributed between daily runs (a maximum of 16 rats could be run at the same time).

Animals' weight was measured daily. There was no difference in weights between genotypes until the end of the experiments (Fig.S2B).

A small number of animals did not go through the last stages of the procedure because of the manifestation of sickness signs, as specified through the number of animals.

Habituation:

Rats were exposed to progressively increased concentrations of EtOH (3%, 6% and 12%). For this, a second bottle, containing the EtOH solution, was placed in animal's home cages in addition to the bottle of water. Each concentration was first introduced for 72h and then for 2h per day during two consecutive days. Each bottle of EtOH was weighed before and after presentation to the animal and no differences in the amount of drinking were found between groups during habituation (data not shown).

Acquisition:

During the first week of acquisition, each press on the AL during the time-in period resulted in the delivery of a drop delivered into a magazine between the two levers (FR-1). Each EtOH delivery was associated with a 10s presentation of a visual cue (light) above the AL. Presses on the IL had no consequences. The following weeks, response requirements were increased to the final value of FR-5

(i.e., animals were required to make five lever presses for each drop delivery). Rats were considered to have acquired stable SA when they pressed the AL more than twice the number of times they pressed the IL, and received a minimum of 5 EtOH deliveries per 1-h session with less than 20% variation in the number of EtOH deliveries earned per session during two consecutive sessions (adapted from [3]).

Progressive Ratio responding:

The response requirement progression was based on the formula $5 \cdot \text{EXP}(0.2 \cdot \text{reinforcement number}) - 5$ [3]. The break point (BP) was defined as the highest ratio completed prior to the first 30min period without a response on the AL. PR sessions lasted a maximum of 3h.

Dose-response curve:

The different doses of EtOH were tested according to a latin-square design, with 3 consecutive sessions for each dose, and two intermediate sessions at the 12% training dose between each testing with another dose.

Extinction:

The criteria for extinction were less than 20 AL presses per session for at least two out of three consecutive days, and less than 20% of variation over the three last consecutive sessions (adapted from [3]).

Reinstatement of EtOH seeking:

The procedures were adapted from previous studies [4,5]. Cue-induced reinstatement test was conducted under conditions identical to that of SA, except that 1) a single presentation of the visual cue (light above the AL for 10s) was delivered response-independently immediately at the start of the session and 2) responses on the AL resulted in contingent presentation of the cue (light above the AL on) and house-light off for 10s, without EtOH delivery.

"EtOH+cue"-induced reinstatement of EtOH seeking consisted of the same conditions of cue-induced reinstatement, except that one single 0.1mL drop of EtOH 12% was delivered into the magazine between both levers concomitantly to the presentation of the visual cue at the start of the session. This condition of EtOH priming is not sufficient to produce a pharmacological effect but has been proposed to recall exteroceptive properties of ethanol [5].

Variable effects of EtOH priming alone (i.e. without the associated cue) have been reported on EtOH seeking in SA procedures, including decreases, mild increases and no modifications in EtOH seeking [6-9]. We have also tested EtOH priming alone-induced reinstatement in similar conditions as those described for extinction except that one single 0.1mL drop of EtOH 12% was delivered into the magazine between both levers at the start of the session or passively orally administered through a pipette just before placing the rats in the operant chambers. However, EtOH-priming alone did not reinstate AL pressing in any group in any condition in our experimental conditions (data not shown).

Relapse indexes were calculated as the subtraction of the number of AL presses averaged for the 3 last extinction sessions from the number of AL presses during the relapse session.

At the end of all reinstatement sessions, the EtOH receptacle was checked for each rat to verify whether there some ethanol was remaining, which was never the case. We also verified in parallel whether a 0.1mL drop of EtOH 12% would still be of similar volume after an hour in similar conditions,

which was the case, to verify that the absence of left ethanol during the experiments was actually due to rat consumption and not to possible evaporation.

Statistical analysis

For two-group comparisons, data were analyzed with unpaired Student's *t* tests or Mann-Whitney U tests when normality and variance homogeneity conditions were not met for parametric test use. Three- and two-way repeated measures ANOVAs were used (group/lever as between- and session/dose/condition as within-subject factors). Significant main effects ($p < 0.05$) were further analyzed using Bonferroni for multiple comparisons post-hoc tests.

Immunofluorescence and c-Fos counting

Brain processing and immunohistofluorescence staining

At the end of the last "EtOH+cue"-induced reinstatement of EtOH seeking or extinction sessions, rats were returned to their home cage for 60min to allow for synthesis and transport of c-Fos proteins to the nuclei of activated neurons. This interval was selected as it is within the period of peak production (between 90 and 120 min) for c-Fos after a specific, initiating event [10]. Animals were injected with a high dose of xylazine/ketamine (13 mg per kg/100 mg per kg, respectively) through intraperitoneal (IP) injections and perfused transcardiacally with 150 mL of phosphate-buffered saline (PBS), followed by 150 mL of 4% paraformaldehyde depolymerized in saline phosphate buffer. The brains were extracted and immersed overnight in the same fixative at 4°C. Sections (50 µm) were cut throughout the entire brain using a vibratome (Leica) and every third slice was processed for immunofluorescence.

Free-floating brain sections were incubated 1h at room temperature in a blocking solution of PBS containing 10% NGS (Abcam LDT, Paris, France) and 0.2% Triton X-100 (Sigma-Aldrich, Saint Quentin Fallavier, France) and then incubated 72h at 4°C with a rabbit anti-c-fos antibody (SantaCruz Biotechnology, Dallas, USA, #sc-52) at 1:1500 dilution in PBS containing 2% NGS and 0.2% Triton X-100. Then, the sections were rinsed with PBS containing 0.2% Triton and then incubated 3h at room temperature with Alexa fluor 488-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch, Ely, UK) at 1:400 dilution in a solution of 2%NGS and 0.2% Triton in PBS. After three rinses in PBS with 0.2% Triton, slices were mounted using Prolong® Gold (Thermofisher Scientific, Villebon-Sur-Yvette, France).

Imaging and quantification

All the images were acquired using a nanozoomer (Hamamatsu Nanozoomer Digital Pathology, 2.0 HT, fluorescence unit option, L11600-05; NanoZoomer's 3-CCD TDI camera, Hamamatsu Photonics; Cellular Imaging Facility of the Institut de la Vision, Paris, France) and analyzed with a 20× objective.

The number of c-Fos positive nuclei was automatically counted with the spot detector module of ICY software (Institut Pasteur, Paris, France). This program allows to select and count cells automatically without experimenter bias (counts were conducted without knowledge of the group assignments). In each coronal section, we sampled both ipsi- and contralateral regions separately and then averaged the density of spots for each structure per animal. The rostro-caudal distance from bregma of the sections used for quantification were (in mm) [11]: -0.80 to +2.70 for cingulate cortex, area 1; -0.80 to +1.7 for cingulate cortex, area 2; +2.2 to + 2.7 for prelimbic and infralimbic cortices; +1.2 to +2.2 for

nucleus accumbens shell and core; -3.60 to -1.60 for paraventricular thalamus; -4.52 to -2.30 for lateral hypothalamus; +1.2 to +1.7 for anterior agranular insula and claustrum. Results were expressed as number of c-Fos positive cell per square millimeter of cerebral tissue.

Statistical analysis

Two-way repeated measures ANOVAs were used (group as between- and condition as within-subject factors). Significant main effects ($p < 0.05$) were further analyzed using Bonferroni for multiple comparisons post-hoc tests. Pearson correlation test was used for correlation analyses between c-Fos and reinstatement levels.

Blood ethanol concentration measurements

We chose to administer EtOH through IP injections over intragastric gavage to limit associated stress, since similar timing patterns were observed in EtOH concentration peak and evolution profile with both methods despite higher EtOH concentrations following IP injections [12]. Drug-naïve rats received an IP injection of EtOH at the concentration of 2 g/kg (volumes injected ranged from 1.5 to 2.3 mL) on an empty stomach before being replaced in their home cages, and were sacrificed at different time points post-injection (15, 30, 90 and 180 minutes) just before blood collection. Serum was extracted through centrifugation at 2000 rpm into Vacutainer BD tubes (Ozyme, Saint-Quentin-en-Yvelines, France) and assayed for EtOH content using an Ethanol Assay Kit based on colorimetric coupled enzyme reaction (MAKO76, Sigma-Aldrich, Saint Quentin Fallavier, France). Blood EtOH concentrations were determined by a standard calibration curve.

Statistical analysis

Two-way ANOVAs were used (group and time as between-subject factors).

Locomotor activity and anxiety-like behavior measurement

An independent batch of drug-naïve rats was exposed to a square open-field measured (l67 cm x L67 cm x H67 cm) for 30 min. The centre of the open-field was defined as the area away from the vicinity of the walls (>11 cm) enclosing the arena. The total distance moved, the mean movement velocity and the % of time spent in the centre were automatically measured with the EthoVision XT tracking software (Noldus, Paris, France). A few days later, rats were placed for 5 min into a dark-light box (l30.5 cm x L25 cm x H47 cm) with two equal-size chambers, one dark with black floor and walls, one brightly lit (500 lux) with white floor and walls, connected by an opening. The % of time spent in the light side, the latency to first entry into the light side and the number of transitions were automatically measured with the EthoVision XT tracking software (Noldus, Paris, France). Some of the files were lost following computer crash, explaining the difference in the number of animals between tests.

Statistical analysis

Data were analyzed unpaired Student's *t* tests.

Food operant self-administration procedure

Sessions were run every day from Monday to Friday and lasted 1h unless specification. Rats were fed 15g and 20g of laboratory standard chow per day from acquisition to the start of extinction, and from

extinction to the end of the experiment, respectively, at the end of the day to favor comparable motivational states between animals at the time of the experimental testing (from 9 am to 4 pm) (except for weekends where animals were fed on Friday evening with adapted food quantities). There was no difference in weights between genotypes until the end of the experiments (Fig.S2C).

Distribution of AL and IL was equally done between groups. Rats from each genotype were equally distributed between daily runs (a maximum of 16 rats could be run at the same time).

Acquisition:

During the first days of acquisition, each press on the AL during the time-in period resulted in the delivery of a food pellet (FR1). Each food pellet delivery was associated with a 10s presentation of a visual cue (light) above the AL. Presses on the IL had no consequences. The next days, response requirements were increased to the final value of FR5. Rats were considered to have acquired stable SA when they pressed the AL more than twice the number of times they pressed the IL, and received a minimum of 80 food deliveries per 1-h session with less than 20% variation in the number of food deliveries earned per session during 2 consecutive sessions. Rats were limited to a maximum of 100 rewards per 1h SA session.

Progressive Ratio responding:

The response requirement progression was based on the formula $5 \cdot \text{EXP}(0.25 \cdot [\text{reinforcement number} + 3]) - 5$, with the first two values replaced by 5 and 10 [13]. The break point was defined as the highest ratio completed prior to the first 30min period without a response on the AL. PR sessions lasted a maximum of 3h.

Extinction:

The criteria for extinction were less than 20 AL presses per session for at least two out of three consecutive days, and less than 20% of variation over the three last consecutive sessions (adapted from [3]).

Reinstatement of food seeking:

Food-induced reinstatement test was done under conditions identical to those of extinction, except that three pellets were automatically delivered into the magazine at the beginning of the session.

Cue-induced reinstatement of food seeking test consisted of the same conditions of the SA sessions, except that 1) a single presentation of the visual cue (light above the AL for 10s) was delivered response-independently immediately at the start of the session and 2) responses on the AL resulted in contingent presentation of the cue (light above the AL) on and house-light off for 10s without food delivery.

Relapse indexes were calculated as the subtraction of the number of AL presses averaged for the 3 last extinction sessions from the number of AL presses during the relapse session.

Statistical analysis

For two-group comparisons, data were analyzed with unpaired Student's *t* tests or Mann-Whitney U tests when normality and variance homogeneity conditions were not met for parametric test use. Three- and two-way repeated measures ANOVAs were used (group/lever as between- and session/condition/treatment as within-subject factors). Significant main effects ($p < 0.05$) were further analyzed using Bonferroni for multiple comparisons post-hoc tests.

SUPPLEMENTARY TABLES

Table S1: ANOVA results for EtOH intake and preference in a two-bottle choice procedure

Parameter	ANOVA type	F value	P value	Figure
% of EtOH preference during acquisition and maintenance	2-way repeated measures	F(session) _{22,594} =42.073 F(group) _{1,27} =0.484 F(sessionXgroup) _{22,594} =2.322	p<0.0001 NS p<0.001	1b
total EtOH intake during acquisition and maintenance	2-way repeated measures	F(session) _{22,594} =10.229 F(group) _{1,27} =0.162 F(sessionXgroup) _{22,594} =2.003	p<0.0001 NS p<0.01	1b
% of EtOH preference during quinine adulteration sessions	2-way repeated measures	F(dose) _{3,78} =40.224 F(group) _{1,26} =0.353 F(doseXgroup) _{3,78} =4.728	p<0.0001 NS p<0.01	1c
% of decrease in EtOH preference during quinine adulteration sessions	2-way repeated measures	F(dose) _{1,26} =19.579 F(group) _{1,26} =6.443 F(doseXgroup) _{1,26} =0.526	p<0.001 p<0.05 NS	1c
Total EtOH intake during quinine adulteration sessions	2-way repeated measures	F(dose) _{3,78} =56.153, F(group) _{1,26} =0.063, F(doseXgroup) _{3,78} =3.868,	p<0.0001; NS p<0.05	1d
% of decrease in EtOH intake during quinine adulteration sessions	2-way repeated measures	F(dose) _{1,26} =28.420 F(group) _{1,26} =8.423 F(doseXgroup) _{1,26} =0.514	p<0.0001 p<0.01 NS	1d
% of EtOH preference during pre-, 1 st and 2 nd post-abstinence re-exposure to EtOH sessions	2-way repeated measures	F(group) _{1,12} =1.914 F(session) _{2,24} =7.033 F(groupXsession) _{2,24} =2.292	NS p<0.01 NS	1e
total EtOH intake during pre-, 1 st and 2 nd post-abstinence re-exposure to EtOH sessions	2-way repeated measures	F(group) _{1,12} =5.155, F(session) _{2,24} =5.263 F(groupXsession) _{2,24} =3.272	p<0.05 p<0.05 NS	1e

Table S2: ANOVA results for EtOH operant self-administration

Parameter	ANOVA type	F value	P value	Figure
Number of AL	3-way	F(group) _{1,58} =7.665	p<0.01	2b

and IL presses during the acquisition of EtOH SA	repeated measures	F(session) _{27,1566} =21.699 F(lever) _{1,58} =58.124 F(groupXlever) _{1,58} =3.046 F(sessionXlever) _{27,1566} =21.669	p<0.0001 p<0.0001 p=0.086 p<0.0001	
total EtOH intake during the acquisition of EtOH SA	2-way repeated measures	F(group) _{1,29} =5.557 F(session) _{27,783} =6.944 F(sessionXinteraction) _{27,783} =0.886	p<0.05 p<0.0001 NS	2b
Dose-response curve of the amount of EtOH consumed during EtOH SA under FR5 schedule of reinforcement	2-way repeated measures	F(group) _{1,29} =1.115 F(dose) _{3,87} =51.724 F(groupXdose) _{3,87} =2.300	NS p<0.0001 p<0.05	2d
Number of AL presses during extinction of previously acquired SA behaviour	2-way repeated measures	F(group) _{1,28} =4.416 F(session) _{18,504} =39.308 F(groupXsession) _{18,504} =0.160	p<0.05 p<0.0001 NS	2e
Number of AL presses during cue-induced EtOH seeking reinstatement	2-way repeated measures	F(group) _{1,28} =2.613 F(cue) _{1,28} =28.329 F(groupXcue) _{1,28} =0.308	NS p<0.0001 NS	2f
Number of AL presses during EtOH+cue-induced EtOH seeking reinstatement	2-way repeated measures	F(group) _{1,25} =14.000 F(EtOH+cue) _{1,25} =61.386 F(groupXEtOH+cue) _{1,25} =6.583	p<0.001 p<0.0001 p<0.05	2g

Table S3: ANOVA results for neuronal activation associated with EtOH+Cue induced reinstatement of EtOH seeking

Parameter	ANOVA type	F value	P value	Figure
Number of AL presses during EtOH+cue-induced EtOH seeking reinstatement in rats sacrificed after a relapse session for c-Fos quantification	2-way repeated measures	F(group) _{1,12} =4.16, NS; F(EtOH+cue) _{1,12} =11.765 F(groupXEtOH+cue) _{1,12} =4.975	NS p<0.01 p<0.05	3a
Number of AL presses during extinction in rats sacrificed after an extinction session for c-Fos quantification	2-way repeated measures	F(group) _{1,8} =12.125 F(session) _{1,8} =3.369 F(groupXsession) _{1,8} =1.946	p<0.01 NS NS	3b
Levels of expression of the c-Fos protein (number of c-Fos-positive cells/mm ²) during EtOH+cue-induced reinstatement of EtOH seeking or extinction	2-way repeated measures	*Cg1: F(group) _{1,17} =0.131 F(relapse) _{1,17} =4.025 F(groupXrelapse) _{1,17} =0.188 *Cg2: F(group) _{1,17} =0.161 F(relapse) _{1,17} =5.353 F(groupXrelapse) _{1,17} =0.691 *PrL: F(group) _{1,18} =1.117 F(relapse) _{1,18} =6.869 F(groupXrelapse) _{1,18} =0.211 * IL: F(group) _{1,18} =3.119 F(relapse) _{1,18} =3.486 F(groupXrelapse) _{1,18} =0.127 *NAcbC: F(group) _{1,18} =0.824 F(relapse) _{1,18} =16.115 F(groupXrelapse) _{1,18} =2.907 *NAcbS: F(group) _{1,17} =0.006 F(relapse) _{1,17} =16.115 F(groupXrelapse) _{1,17} =1.957 *PVTh: F(group) _{1,12} =0.252 F(relapse) _{1,12} =7.267 F(groupXrelapse) _{1,12} =2.317 *LH:	NS NS NS NS p<0.05 NS NS p<0.05 NS NS NS NS NS p<0.05 NS NS p<0.001 NS NS p<0.05 NS	3c

		F(group) _{1,12} =1.504 F(relapse) _{1,12} =6.277 F(groupXrelapse) _{1,12} =0.113	NS p<0.05 NS	
		*Ant. Agranular Insula: F(group) _{1,14} =4.097 F(relapse) _{1,14} =40.791 F(groupXrelapse) _{1,14} =19.583	NS p<0.0001 p<0.001	

Table S4: ANOVA results for EtOH metabolism

Parameter	ANOVA type	F value	P value	Figure
Blood EtOH concentrations at 15, 30, 90 or 180 minutes following an IP EtOH injection	2-way	F(group) _{1,26} =0.950 F(time) _{3,26} =1.641 F(groupXtime) _{3,26} =0.348	NS NS NS	4a

Table S5: ANOVA results for food operant self-administration

Parameter	ANOVA type	F value	P value	Figure
Number of AL and IL presses during the acquisition of food SA	3-way repeated measures	F(group) _{1,80} =0.396 F(session) _{9,720} =280.411 F(lever) _{1,80} =647.068 F(groupXlever) _{1,80} =0.111 F(sessionXlever) _{9,720} =275.406	NS p<0.0001 p<0.0001 NS p<0.0001	5b
Number of AL presses during extinction of previously acquired SA behaviour	2-way repeated measures	F(group) _{1,12} =13.093 F(session) _{12,492} =204.185 F(groupXsession) _{12,492} =1.050	p<0.001 p<0.0001 NS	5d
Number of AL presses during food-induced food seeking reinstatement	2-way repeated measures	F(group) _{1,41} =8.235 F(food priming) _{1,41} =35.392 F(groupXfood priming) _{1,41} =7.732	p<0.01 p<0.0001 p<0.01	5e
Number of AL presses during cue-induced food seeking reinstatement	2-way repeated measures	F(group) _{1,41} =4.446 F(cue) _{1,41} =19.75 F(groupXcue) _{1,41} =5.77	p<0.05 p<0.0001 p<0.05	5f
Number of AL presses during food-induced food seeking reinstatement after saline or nicotine injection	3-way repeated measures	F(group) _{1,39} =8.460 F(food priming) _{1,39} =29.0 F(groupXfood priming) _{1,39} =4.414 F(nicotine) _{1,39} =5.383 F(nicotineXfood priming) _{1,39} =6.20 F(nicotineXgroup) _{1,39} =5.383 F(nicotineXgroupXfood priming) _{1,39} =0.058	p<0.01 p<0.0001 p<0.05 p<0.05 p<0.05 NS NS	5g
relapse index during food-induced food	2-way repeated	F(group) _{1,39} =8.460 F(nicotine) _{1,39} =8.460	p<0.01 p<0.01	5g

seeking reinstatement after saline or nicotine injection	measures	$F(\text{nicotine} \times \text{group})_{1,39} = 0.058$	NS	
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SUPPLEMENTARY FIGURES

Fig. S1

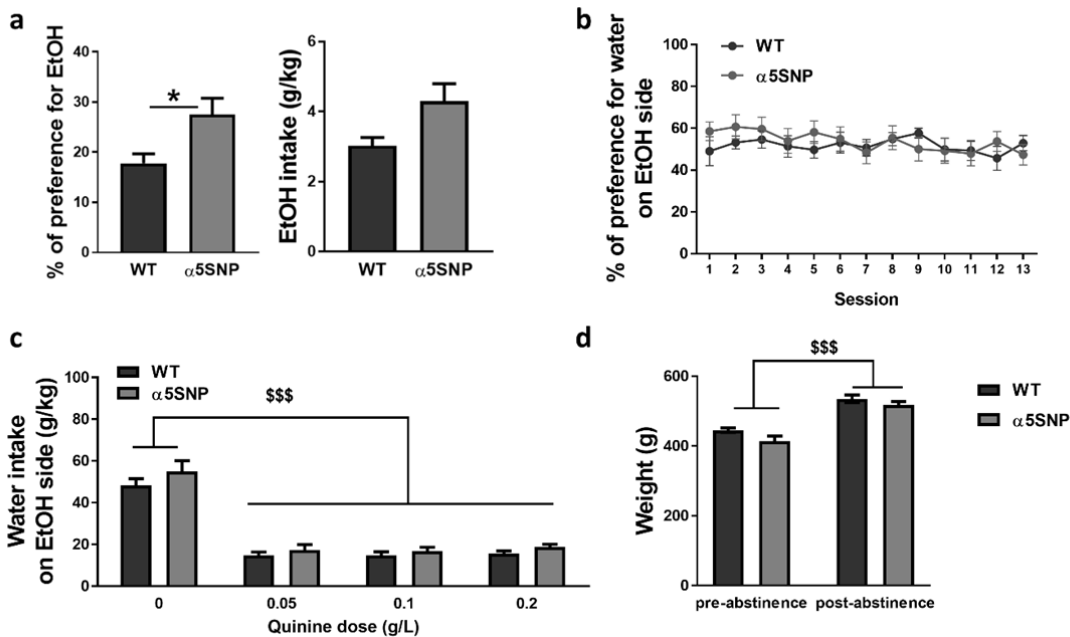


Fig. S1: Ethanol intake and preference during ethanol drinking initiation, water intake and preference during water-only days and animal weights in the two-bottle choice procedure. a % of ethanol (EtOH) preference (left) and total intake (right) during the first choice session in WT (n=15) and $\alpha 5\text{SNP}$ (n=15) rats [Mann-Whitney. $z = -2.509$, $p < 0.05$ for EtOH preference; $z = -1.825$, $p = 0.068$ for EtOH intake]. **b** % of preference, during water-only sessions, for the bottle on the side where EtOH is delivered on choice days, during the acquisition and maintenance phases in WT (n=15) and $\alpha 5\text{SNP}$ (n=14) rats [2-way repeated measure ANOVAs. $F(\text{session})_{13,351} = 0.876$, NS; $F(\text{group})_{1,27} = 0.397$, NS; $F(\text{session} \times \text{group})_{13,351} = 0.709$, NS]. **c** % of water intake, during only-water sessions, into the bottle on the side where EtOH is delivered on choice days, during quinine adulteration sessions in WT (n=8) and $\alpha 5\text{SNP}$ (n=5) rats [2-way repeated measure ANOVAs. $F(\text{dose})_{3,33} = 103.130$, $p < 0.0001$; $F(\text{group})_{1,11} = 3.616$, NS; $F(\text{dose} \times \text{group})_{3,33} = 0.390$, NS [Bonferroni post hoc tests. 0 vs. 0.05g/L: $p < 0.0001$, 0 vs. 0.1g/L: $p < 0.0001$; 0 vs. 0.2g/L: $p < 0.001$]. **d** Weights of WT (n=8) and $\alpha 5\text{SNP}$ (n=6) rats during pre- and first post-abstinence re-exposure to EtOH sessions [2-way repeated measure

ANOVAs. $F(\text{group})_{1,12}=3.079$, NS; $F(\text{time})_{1,12}=428.353$, $p<0.0001$; $F(\text{group}\times\text{session})_{1,12}=2.330$, NS]. Data are mean+s.e.m. Group effect (WT vs. α 5SNP): * $p<0.05$. Session effect (quinine adulteration vs. no quinine or pre-abstinence vs. post-abstinence or post-abstinence) in both groups: \$\$\$ $p<0.001$.

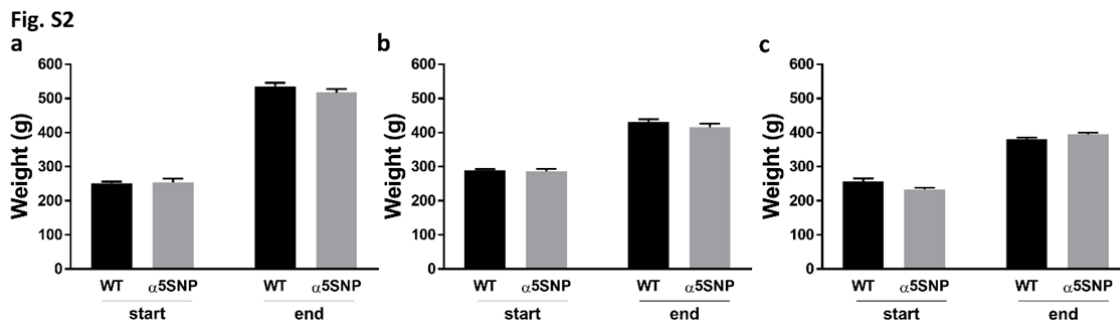


Fig. S2: Rat weights for each experimental group at the start and the end of each experiment. a Weights (g) in WT (start: n=15; end: n=8) and α 5SNP (start: n=15; end: n=6) rats for the two-bottle choice experiment [Unpaired Student's t-test. Start: $t_{28}=0.249$, NS; End: $t_{11}=-1.274$, NS]. **b** Weights (g) in WT (start: n=15; end: n=14) and α 5SNP (start: n=16; end: n=13) rats for the EtOH operant SA experiment [Unpaired Student's t-test. Start: $t_{30}=-0.265$, NS; End: $t_{25}=-1.268$, NS]. **c** Weights (g) in WT (start and end: n=24) and α 5SNP (start and end: n=18) rats for the food operant SA experiment [Unpaired Student's t-test. Start: $t_{41}=-2.008$, NS; End: $t_{41}=1.875$, NS]. Data are mean+s.e.m.

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