Striatal-Enriched Protein Tyrosine Phosphatase Controls Responses to Aversive Stimuli: Implication for Ethanol Drinking

Rémi Legastelois[1¤a], Emmanuel Darcq[1¤b], Scott A. Wegner[1], Paul J. Lombroso[2], Dorit Ron[1]*

1 Department of Neurology, University of California San Francisco, San Francisco, California, United States of America, 2 Yale University School of Medicine, New Haven, Connecticut, United States of America

¤a Current address: R. L. INSERM ERi24, Université de Picardie, Amiens, France
¤b Current address: E. D. Douglas Research Center, McGill University, Montreal, Canada
* dorit.ron@ucsf.edu

Abstract

The STriatal-Enriched protein tyrosine Phosphatase (STEP) is a brain-specific phosphatase whose dysregulation in expression and/or activity is associated with several neuropsychiatric disorders. We recently showed that long-term excessive consumption of ethanol induces a sustained inhibition of STEP activity in the dorsomedial striatum (DMS) of mice. We further showed that down-regulation of STEP expression in the DMS, and not in the adjacent dorsolateral striatum, increases ethanol intake, suggesting that the inactivation of STEP in the DMS contributes to the development of ethanol drinking behaviors. Here, we compared the consequence of global deletion of the *STEP* gene on voluntary ethanol intake to the consumption of an appetitive rewarding substance (saccharin) or an aversive solution (quinine or denatonium). Whereas saccharin intake was similar in STEP knockout (KO) and wild type (WT) littermate mice, the consumption of ethanol as well as quinine and denatonium was increased in STEP KO mice. These results suggested that the aversive taste of these substances was masked upon deletion of the *STEP* gene. We therefore hypothesized that STEP contributes to the physiological avoidance towards aversive stimuli. To further test this hypothesis, we measured the responses of STEP KO and WT mice to lithium-induced conditioned place aversion (CPA) and found that whereas WT mice developed lithium place aversion, STEP KO mice did not. In contrast, conditioned place preference (CPP) to ethanol was similar in both genotypes. Together, our results indicate that STEP contributes, at least in part, to the protection against the ingestion of aversive agents.

Introduction

STriatal-Enriched protein tyrosine Phosphatase (STEP) is a phosphatase that is specifically expressed in the central nervous system (CNS) [1,2]. The *STEP* gene (*PTPN5*) produces alternatively spliced isoforms that include a 46 kDa cytosolic form (STEP$_{46}$) and a 61 kDa membrane-

associated form (STEP$_{61}$) [3]. Both STEP$_{46}$ and STEP$_{61}$ have a wide distribution in the CNS, although STEP$_{61}$ is enriched in striatum and to a lesser extent in lateral amygdala, hippocampus and cortex, while STEP$_{46}$ is expressed in striatum and central nucleus of the amygdala [4]. STEP dephosphorylates and inactivates key neuronal signaling molecules including extracellular signal-regulated kinase1/2 (ERK1/2), stress-activated protein kinase p38 (p38), proline-rich tyrosine kinase 2 (Pyk2), Fyn kinase and the GluN2B subunit of the N-methyl-D-aspartate (NMDA) receptor [5,6,7,8,9,10,11]. As a consequence, STEP opposes the development of synaptic strengthening [11]. STEP is an important regulator of spatial [12] and fear conditioning learning processes [13], as well as motor skills learning and memory [14]. STEP rapidly inhibits p38 signaling after activation by NMDA receptors during learning processes and thereby prevents sustained neuronal excitation and functions as an important neuroprotector [8,15,16].

These studies demonstrate that STEP normally regulates several critical neurophysiological functions. In contrast, alterations of STEP expression and/or function contribute to several neurodegenerative diseases and psychiatric disorders that include Alzheimer's disease (AD), Huntington's chorea, and schizophrenia [11,17,18,19]. STEP was shown to be associated with physiological responses induced by cocaine [20], amphetamine [21] or ethanol [22], and STEP activity or expression is reduced after repeated and intermittent exposure to either stress [23] or ethanol [24]. We recently showed that the intermittent consumption of large amounts of ethanol induces a robust and long-lasting increase in the phosphorylation of STEP$_{61}$ on a specific inhibitory site in the dorsomedial striatum (DMS) of mice, but not in other striatal regions [24]. Furthermore, we showed that knockdown of STEP$_{61}$ specifically in the DMS increased ethanol intake and preference [24].

The development of ethanol drinking behaviors relies in part on the balance between the rewarding and aversive properties of ethanol [25,26]. As our recent data suggested that STEP$_{61}$ inhibition was required for the development of ethanol consumption [24], here, we tested the hypothesis that STEP may modulate the intake of rewarding and/or aversive solutions. Therefore, we determined the consequences of global deletion of STEP on voluntary drinking of ethanol compared to voluntary consumption of sweet and bitter solutions.

Materials and Methods

Materials

Saccharin and quinine hemisulfate were purchased from Sigma (St Louis, MO). Denatonium benzoate was purchased from Alfa Aesar (Ward Hill, MA). Lithium chloride was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Ethics statement

All animal procedures in this report were approved by the University of California San Francisco (UCSF) Institutional Animal Care and Use Committee (AN091738-02G), and were conducted in agreement with the Guide for the Care and Use of Laboratory Animals and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, UCSF).

Animals

Male and female STEP heterozygote mice were obtained from Jackson Laboratories. Pairs of male and female STEP heterozygote mice (C57BL/6 background) were mated in-house to generate STEP WT and KO littermates. Genotypes were determined by RT-PCR analysis of products derived from tail mRNA as described in [21].Male STEP WT and KO mice (2–4 months

at the time of the experiments) were individually housed in a temperature- and humidity-controlled room under a 12-hr light/dark cycle, with food and water available *ad libitum*.

Drugs and treatments

Ethanol solutions for the drinking experiments were prepared from absolute anhydrous ethanol (190 proof) diluted to 3–20% ethanol (*v/v*) in tap water. Saccharin, quinine hemisulfate, and denatonium benzoate were dissolved in tap water. For systemic administrations, lithium chloride was dissolved in saline and absolute anhydrous ethanol was diluted to 20% ethanol (*v/v*) in saline.

Ethanol consumption

Oral ethanol intake was determined using continuous access to ethanol in a two-bottle choice drinking paradigm as previously described [27]. Briefly, drinking sessions were conducted 24 hrs a day, 7 days a week with one bottle containing tap water while the other contained an increasing concentration of ethanol (3, 6, 10 and 20%) with 7 days of access to each concentration. Fresh fluids were provided each time the concentration was changed. The bottles were weighed on days 2, 4, and 7 of each week and the mice were weighed once a week. The position (left or right) of each solution was alternated as a control for side preference.

Saccharin, quinine and denatonium consumption

STEP WT and KO mice were tested for saccharin, quinine and denatonium intake using a continuous access two-bottle choice drinking paradigm. Drinking sessions were conducted 24 hrs a day, 7 days a week with one bottle containing tap water while the other contained an increasing concentration of saccharin (0.005, 0.015, 0.033 and 0.066%), quinine hemisulfate (0.01, 0.03, 0.06, 0.12 and 0.24mM), or denatonium benzoate (0.03, 0.06, 0.12 and 0.24 mM) with 4 days of access to each concentration. Fresh fluids were provided each time the solution was changed. The bottles were weighed every day and the mice were weighed once a week. The position (left or right) of each solution was alternated as a control for side preference.

Conditioned place aversion

The conditioned place aversion procedure was performed according to [28]. The place conditioning boxes (Columbus Instrument) consist of two distinct compartments that differ in color and floor texture. After 5 days of handling and habituation to subcutaneous (s.c.) injections, the initial aversion of STEP WT and KO mice was assessed (preconditioning test). To do so, mice were allowed to freely explore both compartments for 20 min, and the time spent in each compartment was recorded. Three animals that spent more than 70% of the time in either one of the compartments during the preconditioning test were excluded from the study. Treatments were then further counterbalanced between compartments to use an unbiased procedure. The next day, the conditioning training started with two conditioning trials per day for 3 days. Specifically, mice were injected (s.c.) morning (9:00 am) and afternoon (4:00 pm) with either saline (vehicle-paired session) or 130 mg/kg lithium chloride (drug-paired session) and confined in the corresponding-paired compartment for 45 min. Control animals received saline injections mornings and afternoons followed by a 45 min confinement. On the fifth day (postconditioning test), mice had free access to both compartments for 20 min, and the time spent in each compartment was measured.

Conditioned place preference

The conditioned place preference procedure was performed according to [29]. The place conditioning boxes were the same as used for the CPA experiment described above. After 5 days of handling and habituation to intraperitoneal (i.p.) injections, the initial preference of STEP WT and KO mice was assessed (preconditioning test). To do so, mice were allowed to freely explore both compartments for 30 min, and the time spent in each compartment was recorded. One animal that spent more than 70% of the time in either one of the compartments during the preconditioning test was excluded from the study. Treatments were counterbalanced between compartments to use an unbiased procedure. The next day, the conditioning training started with one conditioning trial per day for 8 days. Specifically, mice were administered (i.p.) saline solution and confined in the vehicle-paired compartment for 5 min. The next day, mice were administered (i.p.) ethanol solution (2.0 g/kg) and confined in the ethanol-paired compartment for 5 min. Control animals received saline injections instead of ethanol injections. This schedule was repeated three more times (i.e., for 4 saline- and 4 ethanol-conditioning trials). On the tenth day (postconditioning test), mice had free access to both compartments for 30 min, and the time spent in each compartment was measured.

Locomotor activity

Spontaneous locomotor activity of mice was measured in activity monitoring chambers (43 cm × 43 cm) with horizontal photo beams (Med Associates, St Albans, VT). Horizontal locomotor activity was monitored and the distance traveled (cm) by the mice was recorded for 30 min.

Statistical analysis

Data was analyzed with two-way analysis of variance (ANOVA) or two-way repeated measures-ANOVA (RM-ANOVA). Significant main effects and interactions of the ANOVAs were further investigated with the Student-Newman-Keuls (SNK) *post hoc* test or method of contrast analysis. Statistical significance was set at $p < 0.05$.

Results

STEP controls the consumption of ethanol, quinine and denatonium, but not the consumption of saccharin

We recently showed that the inhibition of $STEP_{61}$ in mice DMS is required for the development of ethanol-drinking behaviors [24]. Specifically, we showed that the voluntary consumption of ethanol induces a robust inhibition of $STEP_{61}$ in the DMS of mice and that knockdown of $STEP_{61}$ in the DMS increased ethanol intake [24]. Consumption is strongly correlated with the rewarding properties of ethanol [30]. However, ethanol intake in both rodents [31] and humans [32,33] is also tempered by their sensitivity to the aversive bitter taste of ethanol. Therefore, we tested whether global deletion of the *STEP* gene in mice leads to changes in the consumption of ethanol (rewarding and bitter [34]), saccharin (rewarding) and quinine and denatonium (aversive) solutions. To do so, STEP WT and KO mice underwent a continuous access to ethanol in a two-bottle choice procedure, during which ethanol concentration was increased every week (from 3% to 20%). Similar to knockdown of $STEP_{61}$ in the DMS [24], STEP KO mice consumed more ethanol compared to their WT littermates (Fig 1A and 1B), whereas total fluid intake remained unchanged (Fig 1C), suggesting that STEP controls ethanol consumption.

Next, we tested the consumption of saccharin and quinine solutions in STEP WT and KO mice in a continuous access two-bottle choice procedure, with the concentration of saccharin (0.005% to 0.066%) or quinine (0.01 mM to 0.24 mM) increasing every four days. As shown in
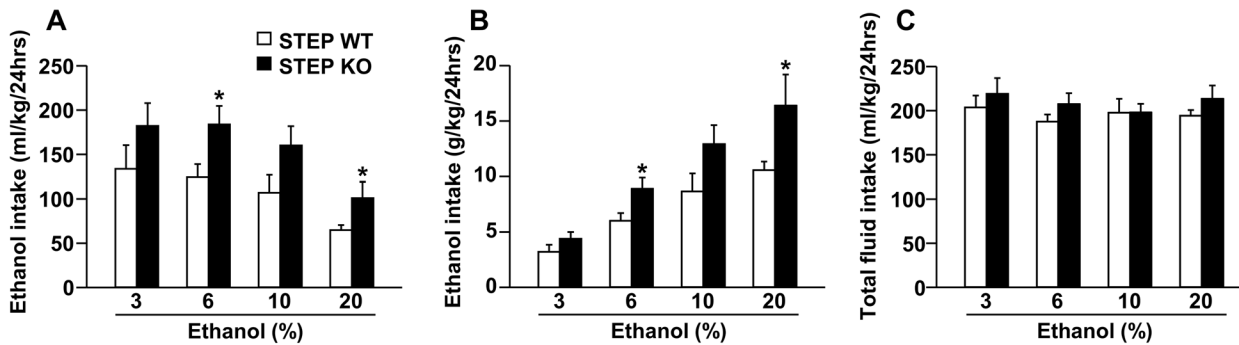
Fig 1. Global deletion of STEP increases ethanol consumption. STEP WT and KO mice were submitted to a continuous access two-bottle choice paradigm with access to one bottle of an ethanol solution and one bottle of tap water. Ethanol concentration was increased every week (3, 6, 10 and 20%) with 7 days of access to each concentration. Results are expressed as mean ± SEM of ethanol consumed expressed in (**A**) ml of solution and (**B**) g of pure ethanol per kg of body weight as well as (**C**) total fluid intake per 24 hours for each ethanol concentration. Two-way RM-ANOVA showed an effect of genotype for **A** [$F_{(1,12)}$ = 4.969, p = .046] **B** [$F_{(1,12)}$ = 5.164, p = .042] but not for **C** [$F_{(1,12)}$ = .954, p = .348], an effect of ethanol concentration for **A** [$F_{(3,36)}$ = 15.565, p <.001], **B** [$F_{(3,36)}$ = 42.103, p <.001] but not for **C** [$F_{(3,36)}$ = .959, p = .423] and no interaction between genotype and ethanol concentration for **A** [$F_{(3,36)}$ = 0.261, p = .853], **B** [$F_{(3,36)}$ = 2.358, p = .088] and **C** [$F_{(3,36)}$ = .469, p = .706]. *p <.05 vs. WT, method of contrasts. n = 6–8.

doi:10.1371/journal.pone.0127408.g001

Fig 2A and 2B, saccharin intake, as well as total fluid intake, was similar in both genotypes at all saccharin concentrations. On the other hand, we found that deletion of the STEP gene disrupted quinine consumption. Specifically, quinine intake was significantly increased at three out of four of quinine concentrations (i.e. 0.01, 0.03 and 0.06 mM) in STEP KO mice compared to WT littermate mice (Fig 3A). Importantly, total fluid intake was similar between both genotypes (Fig 3B). We next tested the drinking of another bitter substance with an unrelated structure, denatonium, in STEP WT and KO mice using a continuous access two-bottle choice procedure, with the concentration of denatonium increased every four days (0.03 mM to 0.24 mM). We found that STEP KO mice drank more denatonium than their WT littermate mice at the denatonium concentrations of 0.03 mM and 0.06 mM (Fig 3C), whereas total fluid intake was unaltered (Fig 3D).
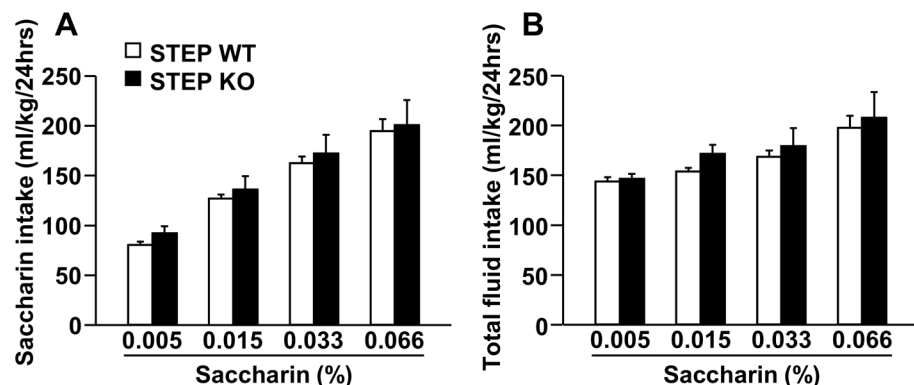

Fig 2. Saccharin consumption is similar in STEP KO and WT mice. STEP WT and KO mice were submitted to a continuous access two-bottle choice paradigm with access to one bottle of a saccharin solution and one bottle of tap water. Saccharin concentration was increased every 4 days (0.005, 0.015, 0.033 and 0.066%). Results are expressed as mean ± SEM of (**A**) saccharin or (**B**) total fluid intake per 24 hours for each saccharin concentration. Two-way RM-ANOVA showed no effect of genotype for **A** [$F_{(1,15)}$ = .357, p = .559] and **B** [$F_{(1,15)}$ = .508, p = .487], an effect of saccharin concentration for **A** [$F_{(3,45)}$ = 72.3, p <.001] and **B** [$F_{(3,45)}$ = 17.207, p <.001] and no interaction between genotype and saccharin concentration for **A** [$F_{(3,45)}$ = .0441, p = .988] and **B** [$F_{(3,45)}$ = .284, p = .837]. n = 8-9.

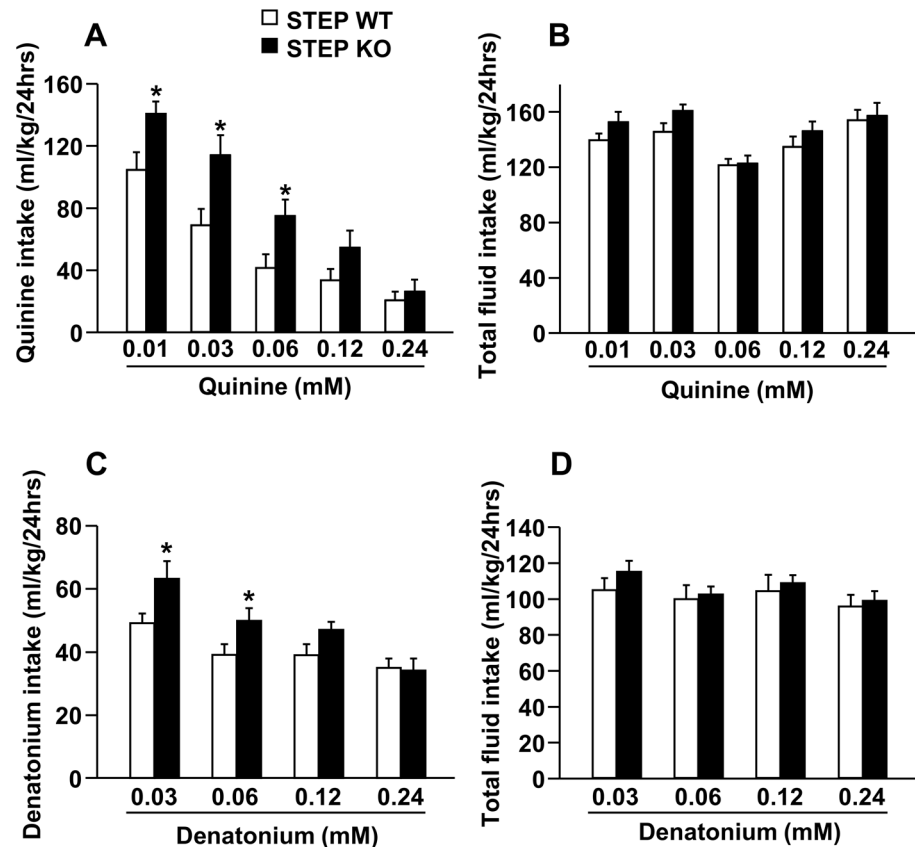doi:10.1371/journal.pone.0127408.g002

Fig 3. Quinine and denatonium consumption is increased in STEP KO vs. WT mice. STEP WT and KO mice were submitted to a continuous access two-bottle choice paradigm with access to one bottle of a quinine or denatonium solution and one bottle of tap water. Quinine or denatonium concentration was increased every 4 days (0.01, 0.03, 0.06, 0.12 and 0.24 mM for quinine and 0.03, 0.06, 0.12 and 0.24 mM for denatonium). Results are expressed as mean ± SEM of (**A**) quinine or (**C**) denatonium intake or total fluid intake per 24 hours for each (**B**) quinine or (**D**) denatonium concentration. Two-way RM-ANOVA showed an effect of genotype for **A** [$F(1,15) = 7.524$, $p = .015$] and **C** [$F(1,16) = 4.594$, $p = .048$] but not for **B** [$F(1,15) = 1.181$, $p = .294$] and **D** [$F(1,16) = 0.365$, $p = .554$], an effect of concentration for **A** [$F(4,60) = 64.307$ $p <.001$], **B** [$F(4,60) = 24.150$, $p <.001$], **C** [$F(3,48) = 19.434$, $p <.001$] and **D** [$F(3,48) = 10.080$, $p <.001$], and no interaction between genotype and concentration for **A** [$F(4,60) = 2.435$, $p = .057$], **B** [$F(4,60) = 1.290$, $p = .284$], **C** [$F(3,48) = 2.462$, $p = .074$] and **D** [$F(3,48) = 1.007$, $p = .398$]. *$p <.05$ vs. WT, method of contrasts. n = 8–9.

doi:10.1371/journal.pone.0127408.g003

We next determined whether the increase in ethanol, quinine and denatonium intake upon deletion of the *STEP* gene was due to alteration in spontaneous locomotor activity. As shown in Fig 4, the distance traveled in an open field was unaltered in STEP KO mice compared to WT mice, indicating that deletion of STEP does not change spontaneous locomotion. Thus, the observed increase in the ingestion of aversive tasting agents such as quinine, denatonium and ethanol is not due to a general increase of spontaneous locomotion. Together, these results suggest that STEP controls the ingestion of aversive tasting agents such as quinine, denatonium and ethanol.

STEP contributes to conditioned place aversion to lithium chloride

Our data thus far suggest that the increase in ethanol intake upon *STEP* gene deletion arises, at least in part, from an increase in the threshold of bitter taste rejection. As bitter taste is an
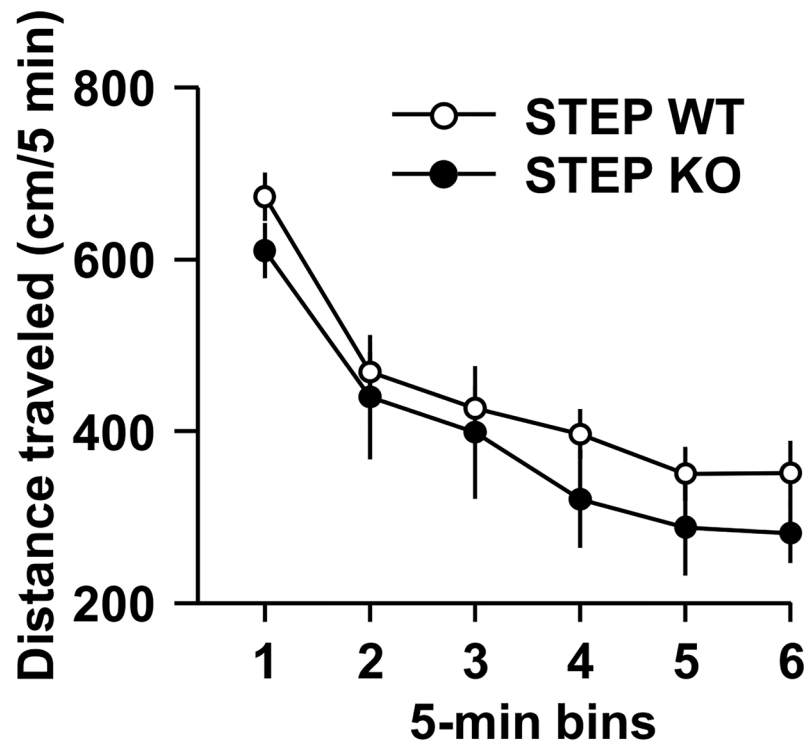
Fig 4. Global deletion of STEP does not alter spontaneous locomotor activity. Results are expressed as mean ± SEM of distance traveled (cm) per 5-min bins in STEP WT and KO mice during a 30-min session. Two-way RM-ANOVA showed an effect of time [$F(5,60) = 49.091$, $p < .001$] but no effect genotype [$F(1,12) = 1.161$, $p = .302$] and no interaction between time and genotype [$F(5,60) = .355$, $p = .877$]. n = 6–8.

doi:10.1371/journal.pone.0127408.g004

aversive stimulus that often signals harmful poisonous substances and is generally avoided by mammals [35,36], we thought to assess whether STEP is involved in the avoidance toward aversive stimuli. To do so, we submitted STEP WT and KO mice to a conditioned place aversion (CPA) paradigm using lithium chloride a substance that when administered systemically causes gastric malaise similar to food poisoning [37]. The procedure consisted of three conditioning sessions to saline and three conditioning sessions to 130 mg/kg lithium chloride as previously described [28]. Whereas WT mice developed a strong aversion to the lithium-paired compartment, STEP KO mice failed to express any avoidance to the environment associated with the aversive stimulus (Fig 5). These results suggest that STEP contributes to the avoidance to aversive stimuli in mice.

Conditioned place preference to ethanol is not altered in STEP KO mice

As ethanol has a bitter taste component [34], we thought that this mechanism could explain, at least in part, the increase of ethanol consumption in STEP KO mice. We therefore tested whether STEP signaling also controls the sensitivity to the pharmacological rewarding properties of ethanol. We used an ethanol-induced conditioned place preference (CPP), which measures the rewarding properties of drugs of abuse [38], in STEP WT and KO mice. The procedure consisted of four conditioning sessions to saline and four conditioning sessions to 2.0 g/kg ethanol as previously described [29]. STEP WT and KO mice expressed a similar CPP to ethanol (Fig 6), suggesting that, under these conditions, STEP does not control the response to ethanol's pharmacological rewarding properties.
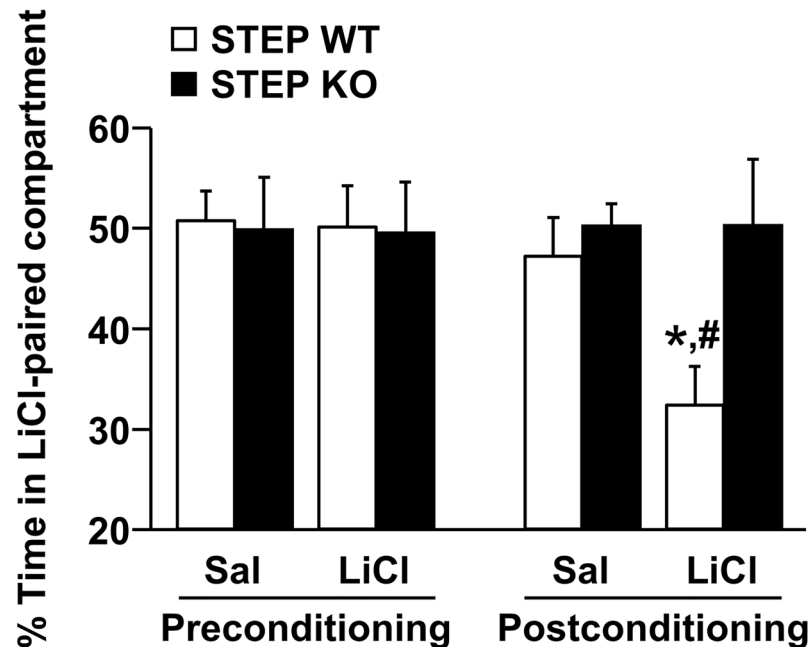
Fig 5. STEP KO mice do not express conditioned place aversion to lithium chloride. STEP WT and KO mice were submitted to a conditioned place aversion paradigm. The conditioning phase consisted in 3 days of daily administration (s.c.) of saline (mornings) or 130 mg/kg lithium chloride (LiCl; afternoons) solution followed by the confinement in the saline- or LiCl-paired compartment for 45 min (3 saline and 3 LiCl conditioning sessions). One day after the third conditioning day, a 20-min postconditioning test was conducted. Results are expressed as mean ± SEM of the percentage of time spent in the LiCl-paired compartment during the preconditioning and postconditioning tests. Two-way ANOVA conducted on the postconditioning values showed an effect of genotype [$F(1,28) = 4.784$, $p = .037$], no effect of conditioning [$F(1,28) = 2.395$, $p = .133$] and no interaction between genotype and conditioning [$F(1,28) = 2.460$, $p = .128$]. *p <.05 vs. Saline, #p <.05 vs. KO, method of contrasts. n = 7–9.

doi:10.1371/journal.pone.0127408.g005

Discussion

We report here that global $STEP_{61}/STEP_{46}$ deletion increased ethanol consumption in mice. We also showed that quinine and denatonium consumption was increased in STEP KO mice compared to WT littermates, whereas saccharin and total fluid intake as well as spontaneous locomotion were unaltered. Our results strongly suggest that STEP controls the consumption of solutions with a bitter taste component such as ethanol, quinine and denatonium. We further showed that mice with global deletion of the *STEP* gene did not develop aversion to the gastric malaise-inducer lithium chloride, although they expressed similar levels of conditioned place preference for ethanol compared to their WT littermates, indicating that STEP plays an important role in the avoidance to aversive stimuli in mice. Altogether, our results suggest a novel mechanism by which STEP participates to the protection against the ingestion of aversive tasting agents like ethanol.

Using a continuous access two-bottle choice of increasing concentration of ethanol, we showed that STEP KO mice drank significantly more ethanol than WT mice, in line with our recent findings [24]. Importantly, it has been shown that ethanol clearance is similar between STEP WT and KO mice [22], ruling out the possibility that the increase in ethanol drinking upon *STEP* gene deletion could be attributable to an enhanced ethanol metabolism, nor was it due to locomotor changes, as spontaneous locomotor activity was similar between both genotypes. Besides the sensitivity to the rewarding properties of ethanol, the perception of its
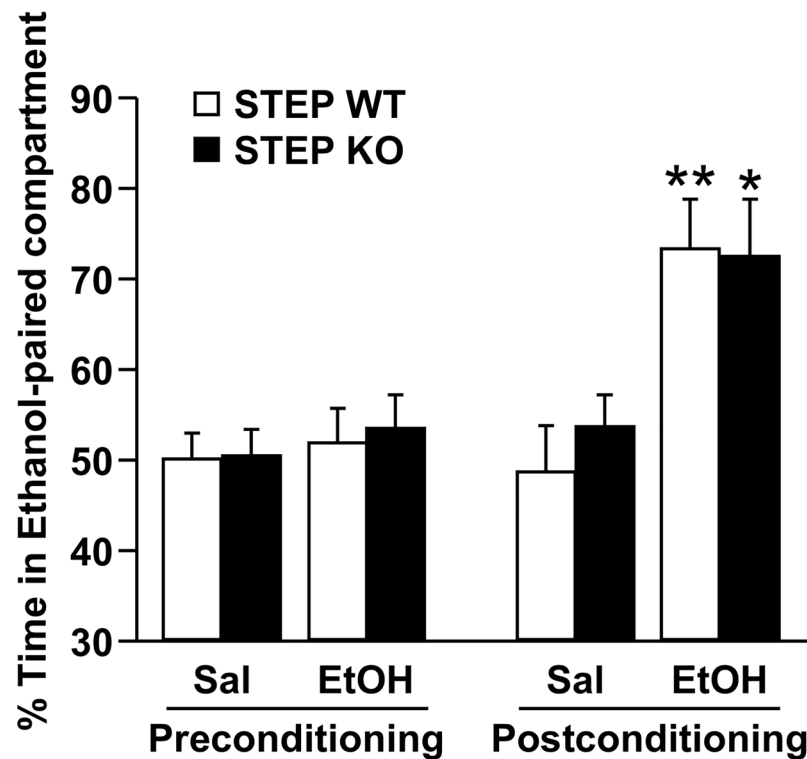
Fig 6. Conditioned place preference to ethanol is not altered in STEP KO mice. STEP WT and KO mice were submitted to an ethanol-induced conditioned place preference paradigm. The conditioning phase consisted in 8 days of daily administration (i.p.) of either saline (Sal) or 2.0 g/kg ethanol (EtOH) solution followed by the confinement in the saline- or ethanol-paired compartment for 5 min (4 saline and 4 ethanol conditioning sessions). One day after the eighth session, a 30-min postconditioning test was conducted. Results are expressed as mean ± SEM of the percentage of time spent in the ethanol-paired compartment during the preconditioning and postconditioning tests. Two-way ANOVA conducted on the postconditioning values showed no effect of genotype [$F(1,33) = .168$, $p = .684$], an effect of conditioning [$F(1,33) = 18.587$, $p <.001.$] and no interaction between genotype and conditioning [$F(1,33) = .330$, $p = .570.$]. *$p <.05$, **$p <.01$ vs. Saline, method of contrasts. n = 8–10.

doi:10.1371/journal.pone.0127408.g006

aversive bitter flavor [34,39] also plays an important role in the levels of voluntary oral consumption of ethanol in both rodents [31] and humans [32,33]. We therefore hypothesized that STEP may promote the avoidance response to solutions with an aversive bitter taste. As STEP KO were aversion-resistant compared to WT littermate mice, our results suggest that STEP is specifically involved in the avoidance to the bitter tastant ethanol. Consistent with our hypothesis, a relationship between the responsiveness to quinine and ethanol has been suggested [40], and a blunted sensitivity to bitter taste may be an important predictor of high ethanol consumption in both rodents [31] and humans [32,33].

To further confirm that STEP contributes to the avoidance to bitter substances, we tested the drinking of another structurally unrelated bitter substance, denatonium, in STEP WT and KO mice. Even though taste receptor cells discriminate between different bitter stimuli [41], rats fail to distinguish quinine from denatonium [42]. Here we showed that STEP KO mice drank more denatonium than WT mice, confirming the involvement of STEP in the avoidance to bitter tastants.

Bitter taste often signals harmful substances and sensitivity to bitter tastants is thus acknowledged to have evolved as a protective mechanism in mammals [35,36]. Therefore, a bitter taste is considered as an aversive stimulus in rodents [43]. To test whether STEP contributes to

the avoidance to aversive stimuli in mice, we used a lithium chloride-induced CPA paradigm. We choose lithium chloride as the aversive stimulus in the CPA experiment because the gastric malaise induced by lithium chloride administration is comparable to food poisoning [37], which can be the result of the consumption of bitter substances [35]. We found that WT mice developed a robust CPA whereas STEP KO mice were resistant to CPA. The fact that comparable ethanol-induced CPP levels were observed between STEP WT and KO mice allowed us to rule out spatial or associative learning deficits as confounding factors in the CPA experiment. These results indicate that STEP is necessary for the development of conditioned aversion and further suggest that inactivation of STEP abolishes the avoidance to aversive stimuli and hence promotes the consumption of aversive tasting agents like ethanol.

We recently demonstrated that inactive $STEP_{61}$ in the DMS increases the drinking of ethanol without altering the palatability of sweet or bitter solutions [24], in apparent contrast with the present report. First, we cannot exclude the occurrence of developmental compensatory mechanism in the mutant mice used herein compared to the gene knock down acutely performed in our previous study. Such compensation may account, at least in part, for the behavioral changes observed in the STEP KO mutant mice. Moreover, it is important to note that herein, both $STEP_{61}$ and $STEP_{46}$ isoforms are deleted in the whole brain, versus specific downregulation of the 61 kDa isoform in the DMS in our previous study [24]. Thus, ethanol-drinking behaviors are driven by different STEP-related mechanisms in several discrete brain regions, some of them regulating the appetence toward the pharmacological effects of ethanol in the DMS and others controlling the aversion to the bitter taste of ethanol. For instance, STEP is expressed in brain regions such as central amygdala and bed nucleus of the stria terminalis [23], both of which are involved in negative affective learning [44,45] and responsive to bitter tastants [46,47]. Further studies will thus be necessary to dissect the contribution of STEP to aversive stimuli in such brain regions.

Recent preclinical reports indicate that STEP inactivation improves cognitive deficits in animal models of AD [19,48], Hence, STEP inhibitors are currently being developed as potential treatments for humans suffering from AD [11,49]. Our previous study [24] and the present report indicate that both specific downregulation of $STEP_{61}$ expression in the DMS and global deletion of the *STEP* gene lead to the increase of ethanol drinking. Therefore, future use of STEP inhibitors in clinical trials should be closely monitored regarding the consumption of alcoholic beverages and the response to aversive, undesirable signals.

Acknowledgments

Author Contributions

Conceived and designed the experiments: RL ED DR. Performed the experiments: RL SAW. Analyzed the data: RL ED. Contributed reagents/materials/analysis tools: PJL. Wrote the paper: RL ED DR.

References

1. Lombroso PJ, Murdoch G, Lerner M (1991) Molecular characterization of a protein-tyrosine-phosphatase enriched in striatum. Proceedings of the National Academy of Sciences of the United States of America 88: 7242–7246. PMID: 1714595

2. Lombroso PJ, Naegele JR, Sharma E, Lerner M (1993) A protein tyrosine phosphatase expressed within dopaminoceptive neurons of the basal ganglia and related structures. The Journal of neuroscience: the official journal of the Society for Neuroscience 13: 3064–3074. PMID: 8331384

3.   Sharma E, Zhao F, Bult A, Lombroso PJ (1995) Identification of two alternatively spliced transcripts of STEP: a subfamily of brain-enriched protein tyrosine phosphatases. Brain research Molecular brain research 32: 87–93. PMID: 7494467

4.   Boulanger LM, Lombroso PJ, Raghunathan A, During MJ, Wahle P, Naegele JR (1995) Cellular and molecular characterization of a brain-enriched protein tyrosine phosphatase. The Journal of neuroscience: the official journal of the Society for Neuroscience 15: 1532–1544.

5.   Nguyen TH, Liu J, Lombroso PJ (2002) Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. The Journal of biological chemistry 277: 24274–24279. PMID: 11983687

6.   Paul S, Nairn AC, Wang P, Lombroso PJ (2003) NMDA-mediated activation of the tyrosine phosphatase STEP regulates the duration of ERK signaling. Nature neuroscience 6: 34–42. PMID: 12483215

7.   Pelkey KA, Askalan R, Paul S, Kalia LV, Nguyen TH, Pitcher GM, et al. (2002) Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. Neuron 34: 127–138. PMID: 11931747

8.   Poddar R, Deb I, Mukherjee S, Paul S (2010) NR2B-NMDA receptor mediated modulation of the tyrosine phosphatase STEP regulates glutamate induced neuronal cell death. Journal of neurochemistry 115: 1350–1362. doi: 10.1111/j.1471-4159.2010.07035.x PMID: 21029094

9.   Pulido R, Zuniga A, Ullrich A (1998) PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. The EMBO journal 17: 7337–7350. PMID: 9857190

10.   Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. Nature neuroscience 8: 1051–1058. PMID: 16025111

11.   Carty NC, Xu J, Kurup P, Brouillette J, Goebel-Goody SM, Austin DR, et al. (2012) The tyrosine phosphatase STEP: implications in schizophrenia and the molecular mechanism underlying antipsychotic medications. Translational psychiatry 2: e137. doi: 10.1038/tp.2012.63 PMID: 22781170

12.   Venkitaramani DV, Moura PJ, Picciotto MR, Lombroso PJ (2011) Striatal-enriched protein tyrosine phosphatase (STEP) knockout mice have enhanced hippocampal memory. The European journal of neuroscience 33: 2288–2298. doi: 10.1111/j.1460-9568.2011.07687.x PMID: 21501258

13.   Olausson P, Venkitaramani DV, Moran TD, Salter MW, Taylor JR, Lombroso PJ (2012) The tyrosine phosphatase STEP constrains amygdala-dependent memory formation and neuroplasticity. Neuroscience 225: 1–8. doi: 10.1016/j.neuroscience.2012.07.069 PMID: 22885232

14.   Chagniel L, Bergeron Y, Bureau G, Massicotte G, Cyr M (2014) Regulation of tyrosine phosphatase STEP61 by protein kinase A during motor skill learning in mice. PloS one 9: e86988. doi: 10.1371/journal.pone.0086988 PMID: 24466306

15.   Deb I, Manhas N, Poddar R, Rajagopal S, Allan AM, Lombroso PJ, et al. (2013) Neuroprotective role of a brain-enriched tyrosine phosphatase, STEP, in focal cerebral ischemia. The Journal of neuroscience: the official journal of the Society for Neuroscience 33: 17814–17826. doi: 10.1523/JNEUROSCI.2346-12.2013 PMID: 24198371

16.   Xu J, Kurup P, Zhang Y, Goebel-Goody SM, Wu PH, Hawasli AH, et al. (2009) Extrasynaptic NMDA receptors couple preferentially to excitotoxicity via calpain-mediated cleavage of STEP. The Journal of neuroscience: the official journal of the Society for Neuroscience 29: 9330–9343.

17.   Gladding CM, Sepers MD, Xu J, Zhang LY, Milnerwood AJ, Lombroso PJ, et al. (2012) Calpain and STriatal-Enriched protein tyrosine phosphatase (STEP) activation contribute to extrasynaptic NMDA receptor localization in a Huntington's disease mouse model. Human molecular genetics 21: 3739–3752. doi: 10.1093/hmg/dds154 PMID: 22523092

18.   Saavedra A, Giralt A, Rue L, Xifro X, Xu J, Ortega Z, et al. (2011) Striatal-enriched protein tyrosine phosphatase expression and activity in Huntington's disease: a STEP in the resistance to excitotoxicity. The Journal of neuroscience: the official journal of the Society for Neuroscience 31: 8150–8162. doi: 10.1523/JNEUROSCI.3446-10.2011 PMID: 21632937

19.   Zhang Y, Kurup P, Xu J, Carty N, Fernandez SM, Nygaard HB, et al. (2010) Genetic reduction of striatal-enriched tyrosine phosphatase (STEP) reverses cognitive and cellular deficits in an Alzheimer's disease mouse model. Proceedings of the National Academy of Sciences of the United States of America 107: 19014–19019. doi: 10.1073/pnas.1013543107 PMID: 20956308

20.   Chiodi V, Mallozzi C, Ferrante A, Chen JF, Lombroso PJ, Di Stasi AM, et al. (2014) Cocaine-induced changes of synaptic transmission in the striatum are modulated by adenosine A2A receptors and involve the tyrosine phosphatase STEP. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 39: 569–578. doi: 10.1038/npp.2013.229 PMID: 23989619

21.   Tashev R, Moura PJ, Venkitaramani DV, Prosperetti C, Centonze D, Paul S, et al. (2009) A substrate trapping mutant form of striatal-enriched protein tyrosine phosphatase prevents amphetamine-induced stereotypies and long-term potentiation in the striatum. Biological psychiatry 65: 637–645. doi: 10.1016/j.biopsych.2008.10.008 PMID: 19026408

22. Hicklin TR, Wu PH, Radcliffe RA, Freund RK, Goebel-Goody SM, Correa PR, et al. (2011) Alcohol inhibition of the NMDA receptor function, long-term potentiation, and fear learning requires striatal-enriched protein tyrosine phosphatase. Proceedings of the National Academy of Sciences of the United States of America 108: 6650–6655. doi: 10.1073/pnas.1017856108 PMID: 21464302

23. Dabrowska J, Hazra R, Guo JD, Li C, Dewitt S, Xu J, et al. (2013) Striatal-enriched protein tyrosine phosphatase-STEPs toward understanding chronic stress-induced activation of corticotrophin releasing factor neurons in the rat bed nucleus of the stria terminalis. Biological psychiatry 74: 817–826. doi: 10.1016/j.biopsych.2013.07.032 PMID: 24012328

24. Darcq E, Ben Hamida S, Wu S, Phamluong K, Kharazia V, Xu J, et al. (2014) Inhibition of striatal-enriched tyrosine phosphatase 61 in the dorsomedial striatum is sufficient to increased ethanol consumption. Journal of neurochemistry. doi: 10.1111/jnc.12701

25. Verendeev A, Riley AL (2013) The role of the aversive effects of drugs in self-administration: assessing the balance of reward and aversion in drug-taking behavior. Behavioural pharmacology 24: 363–374. doi: 10.1097/FBP.0b013e32836413d5 PMID: 23863641

26. Haack AK, Sheth C, Schwager AL, Sinclair MS, Tandon S, Taha SA (2014) Lesions of the lateral habenula increase voluntary ethanol consumption and operant self-administration, block yohimbine-induced reinstatement of ethanol seeking, and attenuate ethanol-induced conditioned taste aversion. PloS one 9: e92701. doi: 10.1371/journal.pone.0092701 PMID: 24695107

27. Yaka R, Tang KC, Camarini R, Janak PH, Ron D (2003) Fyn kinase and NR2B-containing NMDA receptors regulate acute ethanol sensitivity but not ethanol intake or conditioned reward. Alcoholism, clinical and experimental research 27: 1736–1742. PMID: 14634488

28. Darcq E, Befort K, Koebel P, Pannetier S, Mahoney MK, Gaveriaux-Ruff C, et al. (2012) RSK2 signaling in medial habenula contributes to acute morphine analgesia. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 37: 1288–1296. doi: 10.1038/npp.2011.316 PMID: 22218090

29. Lim JP, Zou ME, Janak PH, Messing RO (2012) Responses to ethanol in C57BL/6 versus C57BL/6 x 129 hybrid mice. Brain and behavior 2: 22–31. doi: 10.1002/brb3.29 PMID: 22574271

30. Green AS, Grahame NJ (2008) Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? Alcohol 42: 1–11. doi: 10.1016/j.alcohol.2007.10.005 PMID: 18164576

31. Goodwin FL, Bergeron N, Amit Z (2000) Differences in the consumption of ethanol and flavored solutions in three strains of rats. Pharmacology, biochemistry, and behavior 65: 357–362. PMID: 10683473

32. Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, et al. (2004) Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. Alcoholism, clinical and experimental research 28: 1629–1637. PMID: 15547448

33. Hinrichs AL, Wang JC, Bufe B, Kwon JM, Budde J, Allen R, et al. (2006) Functional variant in a bitter-taste receptor (hTAS2R16) influences risk of alcohol dependence. American journal of human genetics 78: 103–111. PMID: 16385453

34. Blizard DA (2007) Sweet and bitter taste of ethanol in C57BL/6J and DBA2/J mouse strains. Behavior genetics 37: 146–159. PMID: 17096193

35. Glendinning JI (1994) Is the bitter rejection response always adaptive? Physiology & behavior 56: 1217–1227.

36. Garcia J, Hankins WG (1975) The evolution of bitter and the acquisition of toxiphobia. In: Denton DA, Coghlan JP, editors. Olfaction and taste V, Proceedings of the 5th international symposium in Melbourne, Australia. New York: Academic Press. pp. 39–45.

37. Rossi MA, Yin HH (2012) Methods for studying habitual behavior in mice. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al] Chapter 8: Unit 8 29.

38. Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 153: 31–43. PMID: 11255927

39. Scinska A, Koros E, Habrat B, Kukwa A, Kostowski W, Bienkowski P (2000) Bitter and sweet components of ethanol taste in humans. Drug and alcohol dependence 60: 199–206. PMID: 10940547

40. Youngentob SL, Glendinning JI (2009) Fetal ethanol exposure increases ethanol intake by making it smell and taste better. Proceedings of the National Academy of Sciences of the United States of America 106: 5359–5364. doi: 10.1073/pnas.0809804106 PMID: 19273846

41. Caicedo A, Roper SD (2001) Taste receptor cells that discriminate between bitter stimuli. Science 291: 1557–1560. PMID: 11222863

42. Spector AC, Kopka SL (2002) Rats fail to discriminate quinine from denatonium: implications for the neural coding of bitter-tasting compounds. The Journal of neuroscience: the official journal of the Society for Neuroscience 22: 1937–1941.

43. Grill HJ, Norgren R (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neuro-logically normal rats. Brain research 143: 263–279. PMID: 630409

44. Delfs JM, Zhu Y, Druhan JP, Aston-Jones G (2000) Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. Nature 403: 430–434. PMID: 10667795

45. Gracy KN, Dankiewicz LA, Koob GF (2001) Opiate withdrawal-induced fos immunoreactivity in the rat extended amygdala parallels the development of conditioned place aversion. Neuropsychopharmacol-ogy: official publication of the American College of Neuropsychopharmacology 24: 152–160. PMID: 11120397

46. Nishijo H, Uwano T, Tamura R, Ono T (1998) Gustatory and multimodal neuronal responses in the amygdala during licking and discrimination of sensory stimuli in awake rats. Journal of neurophysiology 79: 21–36. PMID: 9425173

47. Park J, Wheeler RA, Fontillas K, Keithley RB, Carelli RM, Wightman RM (2012) Catecholamines in the bed nucleus of the stria terminalis reciprocally respond to reward and aversion. Biological psychiatry 71: 327–334. doi: 10.1016/j.biopsych.2011.10.017 PMID: 22115620

48. Zhang Y, Kurup P, Xu J, Anderson GM, Greengard P, Nairn AC, et al. (2011) Reduced levels of the ty-rosine phosphatase STEP block beta amyloid-mediated GluA1/GluA2 receptor internalization. Journal of neurochemistry 119: 664–672. doi: 10.1111/j.1471-4159.2011.07450.x PMID: 21883219

49. Baguley TD, Xu HC, Chatterjee M, Nairn AC, Lombroso PJ, Ellman JA (2013) Substrate-based frag-ment identification for the development of selective, nonpeptidic inhibitors of striatal-enriched protein ty-rosine phosphatase. Journal of medicinal chemistry 56: 7636–7650. doi: 10.1021/jm401037h PMID: 24083656