Published in final edited form as:

Behav Brain Res. 2007 March 12; 178(1): 70–81.

Strychnine and Taurine Modulation of Amygdala-associated Anxiety-like Behavior is ‘State’ Dependent

Brian A. McCool, Ph.D. and Ann Chappell

Wake Forest University School of Medicine, Dept. Physiology & Pharmacology, Winston-Salem NC 27157

Abstract

Strychnine-sensitive glycine receptors are expressed in many adult forebrain regions, yet the biological function of these receptors outside the spinal cord/brainstem is poorly understood. We have recently shown that rat lateral/basolateral amygdala neurons express strychnine-sensitive glycine-gated currents whose pharmacological and molecular characteristics are consistent with those established for classic ligand-gated chloride channels. The current studies were undertaken to establish the behavioral role, if any, of these strychnine-sensitive glycine receptors. Adult Long-Evans male rats were implanted with guide cannulae targeted at the lateral amygdala and were micro-injected with standard artificial cerebrospinal fluid with or without various doses of strychnine or taurine. Anxiety-like behaviors were assessed with the elevated plus-maze or the light/dark box. In the elevated plus maze, strychnine decreased closed-arm time and increased open-arm time, suggestive of an anxiolytic effect. Similarly, strychnine produced a modest anxiolytic effect in the light/dark box. Post-hoc analysis of ‘open-arm’ time and ‘light-side’ time indicated that aCSF-treated animals were distributed into two apparent groups that displayed either high or low amounts of anxiety-like behavior in a given apparatus. Surprisingly, the pharmacological effects of both strychnine and taurine in these assays were dependent upon a given animal’s behavioral phenotype. Together, these findings are significant because they suggest that the basal ‘emotional state’ of the animal could influence the behavioral outcome associated with drug application directly into the lateral/basolateral amygdala. Furthermore, our findings also suggest that compounds acting at amygdala strychnine-sensitive glycine receptors may actively modulate this basal anxiety-like state.

Keywords

limbic system; glycine receptor; microinjection; plus-maze; light/dark box

Introduction

The amygdala plays a central role in determining the emotional valence of environmental stimuli. To accomplish this, it receives processed sensory information from thalamus and primary somato-sensory cortex, processes this information, and sends projections to brain regions involved with assessing the reward (N. accumbens; [20]) and risks (prefrontal cortex; [17]) associated with the environment. In addition to these more cognitive responses, the amygdala also sends projections to brain areas that regulate an organism’s physical response

Corresponding Author: Brian McCool, Dept. Physiology & Pharmacology, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157, Phone: 336-716-8608, Fax: 336-716-8501, e-mail: bmccool@wfubmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

to environmental stimuli [28]. While this complex functional contribution is necessarily governed by several anatomically, functionally, and morphologically distinct subdivisions, the lateral and basolateral nuclei are known to be centrally important for this integrative process and are the primary input nuclei of this brain area. These subdivisions subsequently regulate the expression of innate anxiety-like behaviors [1] as well as more complex associational learning related to emotionally relevant cues like painful or fear-evoking stimuli [13,18,26, 43]. More recent findings have demonstrated that the lateral/basolateral subdivision also mediate behaviors relevant for drugs of abuse [14,46]. The neurophysiological mechanisms modulating these complex and highly integrative functions are just beginning to be understood. As in many brain regions, the balance between excitatory and inhibitory neurotransmitter systems appears to play an important role in these processes.

Strychnine-sensitive glycine receptors (ssGlyRs) are ligand-gated chloride channels. Like GABA_A receptors, each channel is composed of a complex of five distinct transmembrane-spanning proteins [22] that confer specific biophysical and pharmacologic properties to the receptor complex. These subunits are derived from two distinct gene families, the α and β subunits [21]. The alpha subunit can form functional homomeric channels and can arise from four different gene products (denoted α_{1-4} , reviewed in [8]). However, the presence of the β subunit can confer pharmacological properties that are more consistent with native receptors [19,39]. As its name implies, these chloride-permeant glycine receptors bind the plant alkaloid strychnine with high affinity and are robustly inhibited by this competitive antagonist. This exquisite sensitivity to strychnine distinguishes these channels pharmacologically from the glycine site associated with the N-methyl-D-aspartate family of glutamate receptors. In addition, ssGlyRs can be gated by the β -amino acids, including β -alanine and taurine, which act as partial agonists [45]. Like glycine, taurine and β -alanine are abundant in the central nervous system and could also serve as native agonists for ssGlyRs in some brain regions [34].

In the context of the current study, functional ssGlyR are expressed in adult rat amygdala. When measured in acutely isolated lateral/basolateral amygdala neurons, ssGlyRs possess pharmacological and biophysical characteristics that are consistent with the classical ligand-gated chloride channel [29]. These amygdala ssGlyRs are activated by glycine, β -alanine, and taurine with the latter β -amino acids acting as almost full (β -alanine) or relatively weak (taurine) partial agonists. The amygdala ssGlyRs are also sensitive to low concentrations of strychnine and are completely inhibited by concentrations $<1\mu\text{M}$ [29]. Amygdala glycine receptors are also relatively insensitive to non-competitive antagonist picrotoxin [30], and these findings indicate that amygdala ssGlyRs are likely to heteromeric assemblies containing the β -subunit [39]. These receptors are expressed by both the principal glutamatergic projection neurons as well as some population of GABAergic interneurons and consist primarily of the α_2 subunit-containing channels although mRNAs for α_1 and α_3 are also found in some cells [30]. Exogenous glycine application to amygdala *in vitro* slice preparations produces strychnine-sensitive membrane hyperpolarizations [12]. Since amygdala ssGlyR expression is conserved across several species including humans [16] and non-human primates (B. McCool, unpublished observations), these *in vitro* findings may indicate a highly conserved role for amygdala ssGlyRs.

Despite mounting evidence that ssGlyRs expressed in the adult forebrain can robustly regulate neural function, there are no studies demonstrating a functional role for these receptors *in vivo*. With this in mind, the current study examines the anxiety-related consequences of strychnine and taurine microinjection into rat lateral/basolateral amygdala. We have chosen to quantify anxiety-like behavior with two independent assays, the light/dark box and the elevated plus maze. Both assays provide measures of unconditioned avoidance of naturally aversive environments (i.e. the light-side of the light/dark box or the open-arms of the elevated plus

maze); and, behaviors expressed in these assays frequently display a similar pharmacological sensitivity to anxiolytic compounds. However, the two tests appear to assay unique cognitive and genetic contributions to the expression of anxiety [10,11]. The current experiments will utilize these unique qualities to test the hypothesis that strychnine-sensitive glycine receptors help to modulate amygdala-dependent behaviors.

Materials and Methods

Animals

Adult male Long Evans rats (250g–275g) obtained from Harlan, Indianapolis, IN were used in this study. All rats were individually housed in hanging stainless steel cages with food and water available *ad lib*. Artificial lighting was on for 12 hours/day, starting at 06:00hr. The animals were kept in an AAALAC approved animal facility, with temperature maintained in accordance with NIH Animal Guidelines. All experimental procedures were approved by the Wake Forest University School of Medicine Animal Care and Use Committee.

Surgical Procedure

When each individual weighted between 300g–310g, rats were anesthetized with sodium pentobarbital (50mg/kg IP) and were placed into a rodent stereotaxic instrument (model 962, Kopf Instruments, Tujunga, CA.) with the incisor bar set at 3.3mm below the interaural line. After a midline incision was made over the skull, the skull was scraped clean, four holes were then drilled into the skull and self-tapping stainless-steel screws were inserted to help anchor the guide cannulae. Next, two additional sites were drilled, and stainless steel guide cannulae (26 gauge) of sufficient length were implanted to terminate 1mm above the basolateral amygdala, typically within the lateral nucleus. The coordinates of our target site were: antero-posterior, –2.80 mm from bregma; medio-lateral, from the midline +5.05 mm; ventral from the top of the brain –6.20 mm [36]. Dental cement (Lang Dental, Wheeling, IL) was used to affix the guide cannulae and to stabilize the assembly by adhering to the four stainless steel screws. Obturators were used to maintain the patency of the guide cannulae. Following the surgical procedures, each rat had five days of recovery during which time they were extensively handled by the same experimenter who performed the microinjections.

Microinjection Procedure

Beginning on the sixth day following surgery, each rat was placed in a small holding tub (27×17×12 cm) for four minutes on five consecutive days. During this time, the rats were gently restrained by hand, obturators were removed, and an electric pump was turned on. Following this, clean, sterile obturators were placed back into the guide cannulae. Animals were then placed into a transport cage before being placed back into the home cage. These procedures were performed to habituate rats to the handling and noises associated with the microinjection procedure.

On experimental days, the obturators were removed and 33 gauge injector-cannulae were inserted into the guide cannulae. The injectors were of sufficient length to extend 1mm beyond the end of the guide cannulae. Drug solutions were bilaterally infused for 1 minute with 0.5µl total volume delivered per side. Injectors were left in place for 30 seconds after the end of the infusion and then clean, sterile obturators were inserted back into the guide cannulae. Rats were placed into a transport cage and moved into an adjacent testing room for ten minutes before entering the behavioral apparatus. This time was deemed long enough to allow animals to become accustomed to the testing environment yet short enough to insure that drug-effects were still present during the testing [42].

Apparatus

To assess the effects of drug delivery into the basolateral amygdala, two different assays were employed to assess anxiety-like behaviors. Sessions were performed using the elevated plus-maze [37] with incandescent lighting (~40 lux on the open arms). The apparatus sets atop a 28.5" (72.4 cm)-high aluminum frame (Med Associates, St. Albans, VT). Each runway is 4.0" wide (10.2 cm) and 20.0" long (50.8 cm). Open runways have lips that are 0.5" high (1.3 cm); while closed runways have black polypropylene walls 16.0" high (40.6 cm). Infrared sensors were attached at the opening to each arm to score an animal's entry or exit. Experimental control and data acquisition was achieved with a personal computer interfaced with control units and programmed using MED-PC (Med Associates). Dependent variables included the total amount of time an animal spent in each arm, the total amount of time the animal spent at the central junction, and the number of transitions between arms. At the beginning of each 5 minute session, individuals were placed at the junction of the open and closed arms facing an open arm. Between animals, the apparatus was cleaned with warm water, 70% ethanol, again with warm water, and thoroughly dried.

To measure anxiety with the light/dark box [31], individuals were placed in the 'light' side of a 40cm × 40cm plexiglass arena divided equally into 'light' and 'dark' sides by an opaque plexiglass insert (Rat Truscan Activity Arena, Coulbourn Instruments, Allentown PA) illuminated by ambient room lighting to ~400lux at the center of the 'light' side. The geometric center of the animal ±1.3cm was followed for five minutes by two infrared sensor rings surrounding the entire apparatus, one in the floor plane and one located ~15cm above the floor to measure rearing behavior. Data were collected at 0.25Hz and stored on a personal computer. Dependent variables included total distance moved, total number of moves, total time spent moving, time to leave the light side following initial placement, time to return to the light side after initial entry to the dark side, total time spent in the light and dark compartments, number of light-dark transitions, and number of vertical beam breaks. Between animals, the apparatus was cleaned with warm water, 70% ethanol, again with warm water, and thoroughly dried.

Experimental Design

Animals were randomly assigned to different treatment groups and were bilaterally micro-injected with standard artificial cerebrospinal fluid (aCSF) with or without various doses of strychnine or taurine. Each week, animals were exposed to a single injection followed by testing in a single apparatus. To reduce the 'one-trial tolerance' effect [4] in the various assays, animals exposed to one apparatus on any given week were tested on another apparatus the following week; animals were exposed to any given apparatus only once every three weeks.

A 2×2 or 3×3 Latin-square design was used to assign subjects to an aCSF-injected and strychnine- or taurine-injected groups. For example, during the strychnine studies, animals were divided into two equivalent groups; each group was injected with either aCSF or strychnine (0.5pmol or 167pg) and subsequently exposed to a single behavioral apparatus on the first week of testing. On the second week, aCSF and strychnine 'groups' were switched, injected, and tested on a separate apparatus. This process was repeated until each animal had received both aCSF and drug injections and had been tested on each apparatus.

Taurine experiments were divided into two studies, for the first study, each animal received an aCSF, 50pmol taurine (6.3ng), or 1nmol (125ng) taurine microinjection. In the second study, each animal received aCSF or 5pmol (0.6ng) taurine. Again, injection order and exposure to each apparatus was counterbalanced across weeks such that animals receiving one type of injection and tested on a given apparatus one week would receive a different drug and behavioral assay the next week. We and others have previously shown that repeated microinjections into the same brain region does not substantially influence drug sensitivity

even over several administrations (see [33,42,44]). For all dependent variables examined, the values of aCSF-injected animals across the two experiments did not differ; and these groups were combined for further analysis.

For both the strychnine and taurine studies, there was a significant effect of previous apparatus exposure on the number of vertical-plane entries (rears) and the 'egress latency' in the light/dark box. These behaviors were therefore excluded from analysis. There were no trial-dependent effects for any plus maze dependent variable.

Drugs

Taurine (Tocris, Ellisville, MO) and strychnine-HCl (Sigma Aldrich, St. Louis, MO) were dissolved in artificial cerebrospinal fluid (120mM NaCl, 4.5mM KCl, 1mM MgCl₂, 26mM NaHCO₃, 1.2mM NaH₂PO₄, 10mM D-glucose) that was vigorously bubbled with 95% O₂/5% CO₂ for at least 15minutes. The solutions were then sterile filtered (Millipore 0.2 µm filters, Gellman) and used immediately for injection. For antagonist strychnine, 0.5pmol (167pg) was injected bilaterally. This dose should equate to a local concentration approaching 1µM near the tip of the injection cannula, a maximally efficacious concentration for strychnine-sensitive glycine receptors expressed by lateral/basolateral amygdala neurons [29]. Similarly, taurine doses of 5pmol (0.6ng), 50pmol (6.3ng), or 1nmol (125ng) were injected bilaterally since these doses would approach 10µM, 0.1mM or 2mM near the tip of the injection cannula. These concentrations represent the 'foot' and the 'maximum' of the taurine concentration-response curve that is specific for strychnine-sensitive glycine receptors expressed by the lateral/basolateral amygdala neurons [29].

Histology

Following all behavioral assays, rats were given a lethal dose of sodium pentobarbital (90 mg/kg, IP) and, when deeply anesthetized, were transcardially perfused with buffered saline, followed by 10% formalin in saline. The brains were removed and stored in 10% formalin for at least 1 week before being sectioned on a freezing microtome (60 µm sections), mounted onto slides, and stained with cresyl violet according to standard procedures. The location of the guide cannulae was determined using a light microscope. The approximate tip of the guide cannulae in each rat is shown in Figure 1.

Data Analysis

All analyses and fits of data were performed using Prism 4.02 (GraphPad Software Inc, San Diego CA). For figures 3–7, data were normalized to the mean values for aCSF-controls within a particular experiment and expressed as 'percent'. Where appropriate, dependent variables were analyzed using paired t-test/paired two-way ANOVA (strychnine) or standard between-group two-way ANOVA (taurine) according to established procedures [35]. Tests for normality were performed using the Shapiro-Wilk test [47]. $P < 0.05$ was considered significant in all cases.

Results

Strychnine Microinjection into the Amygdala Reduces Anxiety-like Behavior

Consistent with previous results demonstrating that lateral/basolateral amygdala neurons express functional strychnine-sensitive glycine receptors [12,29,30], microinjection of strychnine (0.5pmol or 167pg) directly into this brain region reduced 'anxiety-like' behaviors in two separate assays. In the elevated plus maze (Table 1), strychnine injected into the basolateral amygdala significantly decreased the amount of time rats ($n=21$, two separate cohorts) spent in the closed arms ($P < 0.05$, paired t-test) and also tended to increase ($P \sim 0.1$,

paired t-test) open-arm time. However, strychnine did not substantially alter the number of open-arm entries or the total number of arm entries. Neither was the amount time animals spent in the junction between open- and closed-arms significantly affected by strychnine microinjection (not shown).

Because different assays of ‘anxiety-like’ behavior appear to measure distinct behavioral components [23], we confirmed the apparent anxiolytic effects of strychnine using the light/dark box (Table 1). As in the plus maze, strychnine injected into the basolateral amygdala caused modest effects on anxiety-like behavior suggested by increased number of transitions between the light and dark compartments ($P<0.05$, paired t-test) and the reduced latency to re-enter the light-side following initial egress ($P<0.05$, paired t-test). There was also a trend for strychnine to increase the amount of time spent in the light-side of the apparatus ($P\sim 0.1$, paired t-test). The time the rats spent moving in the apparatus (235.5 ± 3.7 sec for aCSF vs. 236.3 ± 3.2 sec for strychnine) and the total number of moves made during the assay (75.5 ± 2.6 for aCSF vs. 73.9 ± 2.3 for strychnine) were not significantly affected. Together, these findings indicate that strychnine, an antagonist of glycine receptors expressed in the basolateral amygdala, modulates anxiety-like behavior expressed in both the elevated plus-maze as well as the light/dark box.

Taurine Microinjection into the Amygdala and Anxiety-like Behavior

In light of our findings with strychnine, we hoped to examine the effects of a glycine receptor agonist on anxiety-like behaviors. Because the amino acid glycine is also a co-agonist for the NMDA receptor [25], we chose to inject taurine since this β -amino acid is a partial agonist with some efficacy at amygdala strychnine-sensitive glycine receptors [29] and does not possess any apparent co-agonist activity at the NMDA receptor [3,32]. Taurine microinjections were performed across two separate experiments that utilized different doses. For experiment 1 ($n=12$), we microinjected aCSF, 50pmol (6.3ng) taurine, and 1nmol (125ng) taurine while experiment 2 ($n=13$) tested aCSF and 5pmol (0.6ng) taurine.

In the elevated plus maze, neither open-arm time, closed-arm time, nor open-arm entries were significantly different between experiments; aCSF-injection data was combined from the two different experiments. Regardless, data from both experiments demonstrated that taurine did not produce a dose-dependent effect on any plus-maze dependent-variables (Table 2). In support of these findings, taurine microinjection into the amygdala also did not have any significant effects in the light dark box (Table 2). These findings suggest that the effects of taurine on anxiety-like behavior are negligible across this population of animals.

Rat Behavior in the Plus Maze and Light/Dark Box can be segregated into Distinct Phenotypic Populations

During the analysis of the previous datasets, it was noted that the standard error associated with many of the ‘anxiety’-specific dependent variables in the aCSF-injected animals did not decrease even after the addition of substantial numbers of rats to the study. This suggested to us that the data may not be normally distributed about a single mean. Indeed, the distribution of open-arm times from aCSF-injected rats on the plus-maze was found not to be normal ($W=0.92$, $P<0.01$, $n = 45$ for strychnine and taurine studies). A plot of this distribution (Figure 2A) suggested that the data were consistent with two separate populations, animals that avoided the open-arm (mean time of ~ 30 sec) and those with longer open-arm times (mean ~ 120 sec). The other plus-maze dependent variables, including open-arm entries, closed-arm time, and junction time, appeared to be described as normal distributions (not shown).

These findings led us to examine the light/dark box distributions in aCSF-injected rats. There are several dependent variables in this assay that are believed to be measures of ‘anxiety’ in

rodents, including total time spent in the light-side, number of light/dark transitions, and time to re-enter the light-side once the animal has made its initial egress into the dark. The light/dark transitions in aCSF-injected animals was normally distributed ($W=0.98$, $P>>0.05$) while the 'reentry latency' (time to re-enter the light-side following initial egress into the dark) distribution was not ($W=0.68$, $P<0.0001$). In the latter case, the distribution appeared biased toward short latencies ($<60\text{sec}$) for the majority of animals ($n=36/45$) with a small population ($9/45$) taking much longer ($>120\text{sec}$) to re-enter the light-side (not shown). The light-side time distributions (Fig. 2B) were in contrast spread across a range and could be well-fit by single- ($R^2=0.68$) or two-component ($R^2=0.87$ and 0.94 for each) Gaussian distributions. Together with the plus-maze finding, our data suggest that aCSF-injected animals can be subdivided into at least two populations possessing either high or low levels of anxiety-like behaviors.

'Phenotype' Influences Strychnine Effects in the Plus Maze and Light/Dark Box

We subsequently re-analyzed the plus maze and light/dark box data by first arbitrarily separating animals into groups based on their relative expression of anxiety-like behaviors. For the plus maze, animals were segregated into 'low open-arm time' or 'high open-arm time' phenotypes based on their apparent open-arm time when injected with aCSF (Fig. 2A). 'Low' animals were so identified if their open-arm times were $<90\text{sec}$ while 'High' animals were defined as having open-arm times $\geq 90\text{sec}$. This time point represents the approximant gap between the two populations identified in Figure 2A and generally divided each experimental group in two approximately equivalent populations. Two-way ANOVA analysis revealed a significant interaction between 'phenotype' and strychnine treatment for closed-arm time ($F=7.2$, $P<0.05$) and trend towards significance for open-arm time ($F=3.5$, $P=0.08$). A Bonferroni's post-test of this data indicated that strychnine significantly increased open-arm times (Fig. 3A, $P<0.05$) and decreased closed-arm times (Fig. 3B, $P<0.01$) in 'low open-arm time' animals but did not affect 'high open-arm time' individuals. Neither junction-time (Fig. 3C) nor open-arm entries (Fig. 3D) was significantly altered by strychnine microinjection. However, 'low-open arm time' animals had significantly fewer open-arm entries than 'high-open arm time' individuals (Fig. 3D, $F=14.84$, $P<0.01$). These findings suggest that the segregation of animals based on open-arm times may in fact predict the efficacy of strychnine microinjection into the basolateral amygdala and that the population-effects of strychnine (Table 1) were due almost entirely to the drug's actions in 'low open-arm time' individuals.

For the light/dark box, data were segregated based on light-side times. However, since two distinct populations could not be reliably identified (Fig. 2B), we used the mean light-side time for each experiment to divide animals into two groups, 'low light-side time' animals representing the half-population spending the least amount of time in the light-side and 'high light-side time' individuals that spent more of their time in the light-side. Two-way ANOVA across phenotype and strychnine treatment revealed a significant interaction between variables for light-side time (Fig. 4A; $F=10.6$, $P<0.01$). Bonferroni posttests of these data indicate that strychnine injection significantly increased in light-side time in 'low light-side time' animals ($P<0.01$) without affecting 'high light-side time' individuals. The number of transitions between the light and dark compartments, a measure of exploratory behavior, was significantly different between phenotypes (Fig. 4B; $F=11.6$, $P<0.01$). However, unlike the time spent in the light-side, only the number of transitions of 'high light-side time' animals appeared to be influenced by strychnine microinjection ($P<0.05$). Strychnine also significantly decreased the re-entry latency but only for the 'low light-side time' animals (Fig. 4C; $P<0.05$ with Bonferroni's post-hoc). There was also a trend for the different phenotypes to differ in this particular dependent variable ($F=3.5$, $P\sim 0.07$). Move distance was not significantly altered by strychnine microinjection in either phenotype (Fig. 4D). These findings indicate that strychnine microinjection into the lateral/basolateral amygdala specifically alters many of the variables ascribed to anxiety-like behavior. Like the plus-maze data, the 'basal' (e.g. aCSF-injected)

anxiety-like behavior of an animal may be associated with strychnine efficacy across several distinct light/dark box variables.

'Phenotype' Also Influences Taurine Efficacy in the Plus Maze but not the Light/Dark Box

As with strychnine, we re-analyzed the taurine microinjection data by segregating animals based on their apparent 'basal' (aCSF) phenotype. In this context, there was a significant effect of both taurine dose and phenotype on open-arm time (Fig. 5A; $F=3.6/P<0.05$ for taurine effect and $F=10.8/P<0.01$ for phenotype, repeated-measures two-way ANOVA) with Bonferroni's posthoc analysis revealing a significant increase in open-arm time at the 50pmol (6.3ng) dose but only in 'low open-arm time' animals. This suggested an anxiolytic effect of taurine that was further supported by significant increases in the number of open-arm entries in these same animals (Fig. 5D; $F=4.9$, $P<0.01$ for an overall effect of taurine dose). Bonferroni posttests of these open-arm entry data identified a significant increase for both the 50pmol dose ($P<0.01$, **) in the 'low open-arm time' animals but not in the 'high open-arm time' phenotype. There was a trend from the 1nmol (125ng) taurine dose to produce similar effects on open-arm time and entries although this did not reach statistical significance. Similar non-significant trends were seen for decreased closed-arm time in the 'low' animals (Fig. 5B). Taurine did not substantially affect the total number of arm entries in either phenotype (not shown) nor did it modulate the amount of time animals spent in the junction between open and closed arms (Fig. 5C). Together, these data suggest that microinjection of taurine into the lateral/basolateral amygdala can alter some dependent variables measured in the plus maze. In contrast to these data, taurine did not produce substantial effects for any variable measured in the light/dark box (Fig. 6).

Relationship between Plus-Maze and Light/Dark Box Populations

Our data clearly suggest that ligands acting at BLA glycine receptors are most effective in animals exhibiting relatively high levels of anxiety-like behavior. However, the reduced efficacy of taurine relative to strychnine, particularly in the light/dark box, led us to examine whether individuals with high levels of anxiety-like behavior in one apparatus were the same individuals exhibiting similar behaviors in the other apparatus. Using aCSF data from animals in both the strychnine and taurine studies ($n=45$), correlation analysis of light-side time in the light/dark box and open-arm time in the plus maze indicated a significant co-variation between these dependent variables (Spearman rank $r_s = 0.52$ with a 95% confidence interval = 0.26 to 0.71; $P<0.001$). However, these aCSF-injected animals spent $47.8\pm4.4\%$ of the time in the light-side of the light/dark apparatus while spending only $21.4\pm3.1\%$ of their time on the open-arms of the plus maze. Thus, while there is significant co-variation between these measures, these data also imply that anxiety-like behaviors, as measured by these dependent variables, are not as robustly expressed in the light/dark box compared with the plus maze.

To examine how well populations defined by one assay might predict drug sensitivity in the other assay, we performed a posthoc segregation of aCSF-injected animals based on their light-side times in the light-dark box (these are the 'low light-side time' and 'high light-side time' animals illustrated in Figures 4 & 6) and then assessed the plus maze effects of BLA microinjection of strychnine and taurine in these specific individuals. This effectively examines drug sensitivity in animals that found both behavioral assays anxiogenic relative to the entire population. For strychnine-injected animals, there were significant main effects for strychnine ($F=6.0$, $P<0.05$) and for the interaction between strychnine and phenotype ($F=10.1$, $P<0.01$, two-way ANOVA). This was due exclusively to the increased amount of time 'low light-side time' individuals ($n=9$) spent in the open arm of the plus maze following strychnine microinjection (Fig. 7A; $P<0.01$ with Bonferroni posttests). This strychnine-sensitive increase in open-arm time in 'low light-side time' animals was associated with significantly decreased closed-arm times (not shown; $P<0.01$ from Bonferroni's posttest) with main effects of

strychnine ($F=8.8$, $P<0.01$) and interaction between phenotype and strychnine ($F=5.8$, $P<0.05$). Strychnine microinjection did not produce any effects on junction time (no shown), on the number of arm transitions (not shown), or on open-arm entries (Fig. 7B) regardless of phenotype.

Similar results were found with the taurine microinjection dataset. That is, there was a significant interaction between taurine and phenotype for light-side time ($F=3.7$, $P<0.05$). Posttests indicated a significant increase in open-arm time in the light/dark-defined 'low light-side' individuals (Fig. 7C) at the highest dose of taurine tested (1nmol or 125ng; $P<0.05$). Regardless of the population, neither closed-arm time (not shown) nor junction time (not shown) nor open-arm entries (Figure 7D) were significantly influenced in a dose-dependent fashion by taurine microinjections into this brain region. Together, these data strongly suggest that the populations defined by anxiety-like behaviors expressed in the light-dark box are differentially sensitive to the apparent anxiolytic activity of BLA-administered strychnine and taurine when measured in the plus maze.

Discussion

There are several major findings associated with the current work. Most importantly, strychnine and, to some extent, taurine produce behavioral effects in both elevated plus maze and the light/dark box when microinjected into the lateral and basolateral amygdala. These data may suggest that the strychnine-sensitive glycine receptors identified using *in vitro* preparations of this brain region [12,29] function to modulate anxiety-like behaviors *in vivo*. Previous work has shown that lateral/basolateral amygdala GABA_A receptor activation, either directly with agonist [9] or indirectly with benzodiazepine agonist [51], causes anxiolysis while inhibition with receptor antagonists cause anxiogenesis [44]. Given that both glycine and GABA_A receptors are ligand-gated chloride channels, we initially hypothesized that glycine receptor activation by taurine would also produce anxiolytic effects while inhibition of these receptors by strychnine might produce anxiogenic effects if the receptors were active under the conditions of our behavioral assays. Since the current study demonstrates that the glycine receptor antagonist strychnine actually increased the expression of anxiety-like behavior, we must conclude that glycine and GABA_A receptors expressed in the lateral/basolateral amygdala do not have similar neurophysiological roles despite their similar biophysical characteristics.

The similarities between the anti-anxiety profiles for the partial agonist taurine and the competitive antagonist strychnine were unexpected and deserve some discussion. One possible explanation would be that taurine and strychnine are targeting separate neurotransmitter systems. At highest dose used here, taurine (a ~2mM concentration equivalent) could theoretically activate a modest GABA_A receptor-mediated current. This dose might therefore produce an initial decrease in neuronal activity and could reduce anxiety-like behavior as is seen with GABA_A receptor agonists like muscimol [9]. Although we observed such an anxiolytic effect in our studies, the lower 50pmol (6.3ng) dose produced similar effects but would be expected to produce a 0.1mM concentration equivalent near the injection site, well below that needed to activate either recombinant [15] or native [48] GABA_A receptors. Thus, while we can not conclusively exclude possible contributions by GABA_A receptors, it would be difficult to imagine an exclusively GABAergic mechanism to explain our taurine data given the similar effects of 50pmol (6.3ng) and 1nmol (125ng) taurine. An alternative explanation might be that taurine is producing its behavioral effects through BLA strychnine-sensitive glycine receptors, but acting as an antagonist rather than partial agonist. In isolated lateral/basolateral amygdala neurons, taurine has only poor-to-modest efficacy compared to glycine [29]. Notably, several studies on recombinant glycine receptors have suggested that taurine possesses both 'antagonist-like' and 'agonist-like' activity with the antagonist-interactions occur at a greater relative potency [45,49]. Although similar studies have not been performed

on native receptors, the relatively potent, dose-dependent effect of taurine in the current study along with the behavioral similarities between taurine and strychnine microinjection into the BLA are both consistent with idea that taurine may be acting more like an antagonist at strychnine-sensitive glycine receptors, at least in 'anxious' animals.

One of the most surprising findings of the current study is that segregation of animal phenotypes appeared to influence the efficacy of each drug. Our post-hoc segregation is none-the-less consistent with the natural separation of behavioral phenotypes in both rodent and non-human primate studies of anxiety-like behavior. For example, the elevated plus maze can segregate Wistar rats into populations with distinct levels of anxiety-like behavior [6]. And, genetic polymorphisms in the serotonin transporter are associated with differential expression of anxiety-related behaviors in non-human primates [5]. There are also several examples of phenotype-specific effects of anxiolytic drugs. The neurosteroid dehydroepiandrosterone is more efficacious in rats exhibiting heightened 'behavioral despair' [38]. And, distinct inbred mouse strains that differ in their 'anxiety' phenotypes are differentially sensitive to the anxiolytic effects of diazepam [23]. Such 'population'-sensitivity appears to also be common across species as well [27]. The differential sensitivity of the individual phenotypes to the actions of both strychnine and taurine may therefore have much broader implications for the role of ssGlyR in the adult rat forebrain.

Finally, there were test-specific effects of drugs that deserve additional consideration. While strychnine appeared to reduce anxiety-like behavior in both assays, the most apparent effects of taurine were anxiolytic in the plus maze but negligible in the light/dark box. If glycine receptor activation is indeed associated with increased anxiety-like behavior as was suggested by our strychnine findings, the differential effects of taurine in these assays may reflect the relative differences in glycine receptor activity in each assay. Of course, assay-dependent drug effects may also reflect distinct neurobiological contributions to anxiety-like behaviors expressed in each apparatus. Clearly, the plus maze and light/dark box share many characteristics. They are both perceived as unconditioned responses to naturally aversive environments; and, anxiety-like behaviors expressed in both assays are sensitive to many of the same pharmacological manipulations (reviewed in [2,7,41,50]). Despite these similarities, anxiety-like behavior is clearly multidimensional [40]; and, the unique environments in each assay are likely to recruit unique neurobiological processes. This is perhaps best demonstrated by the unique pharmacological sensitivities of behaviors expressed in the plus maze and light/dark box that have been occasionally described. For example, benzodiazepine and serotonin-specific (e.g. 5HT_{1A}) anxiolytics do not always present a consistent anxiolytic profile when tested in both assays [10,24]. Thus, the test-specific effects of taurine may merely provide an additional demonstration of the complex and multidimensional character of anxiety.

In conclusion, the data presented here suggests that strychnine- and taurine-sensitive receptors expressed in the lateral/basolateral amygdala may help modulate anxiety-like behaviors in rodents. More importantly, the behavioral influence by these receptors appears to be specific for 'sensitive' individuals. These findings suggest that heightened anxiety-like states are associated with increased strychnine-sensitive receptor activity and thus increased sensitivity to their pharmacological manipulation.

Acknowledgements

We thank Drs. Hank Samson and Dustin DuBois for their support and constructive comments on this manuscript. This work was funded by in part by AA014445 and AA015179.

References

1. Amaral DG. The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. *Biol Psychiatry* 2002;51:11–7. [PubMed: 11801227]
2. Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* 2001;125:141–9. [PubMed: 11682105]
3. Benavides J, Rivy JP, Carter C, Scatton B. Differential modulation of [3H]TCP binding to the NMDA receptor by L-glutamate and glycine. *Eur J Pharmacol* 1988;149:67–72. [PubMed: 2840302]
4. Bertoglio LJ, Carobrez AP. Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. *Behav Brain Res* 2000;108:197–203. [PubMed: 10701663]
5. Bethae CL, Streicher JM, Coleman K, Pau FK, Moessner R, Cameron JL. Anxious behavior and fenfluramine-induced prolactin secretion in young rhesus macaques with different alleles of the serotonin reuptake transporter polymorphism (5HTTLPR). *Behav Genet* 2004;34:295–307. [PubMed: 14990868]
6. Borta A, Schwarting RK. Inhibitory avoidance, pain reactivity, and plus-maze behavior in Wistar rats with high versus low rearing activity. *Physiol Behav* 2005;84:387–96. [PubMed: 15763576]
7. Bourin M, Hascoet M. The mouse light/dark box test. *Eur J Pharmacol* 2003;463:55–65. [PubMed: 12600702]
8. Breiting HG, Becker CM. The inhibitory glycine receptor-simple views of a complicated channel. *Chembiochem* 2002;3:1042–52. [PubMed: 12404628]
9. Bueno CH, Zangrossi H Jr, Viana MB. The inactivation of the basolateral nucleus of the rat amygdala has an anxiolytic effect in the elevated T-maze and light/dark transition tests. *Braz J Med Biol Res* 2005;38:1697–701. [PubMed: 16258641]
10. Chaoulloff F, Durand M, Mormede P. Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. *Behav Brain Res* 1997;85:27–35. [PubMed: 9095339]
11. Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ, Maxson SC, Miner LL, Silva AJ, Wehner JM, Wynshaw-Boris A, Paylor R. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl)* 1997;132:107–24. [PubMed: 9266608]
12. Danover L, Pape HC. Strychnine-sensitive glycine responses in neurons of the lateral amygdala: an electrophysiological and immunocytochemical characterization. *Neuroscience* 1998;85:427–41. [PubMed: 9622242]
13. Davis M, Rainnie D, Cassell M. Neurotransmission in the rat amygdala related to fear and anxiety. *Trends Neurosci* 1994;17:208–14. [PubMed: 7520203]
14. Di Ciano P, Everitt BJ. Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology* 2004;47 :202–13. [PubMed: 15464138]Suppl 1
15. Dominguez-Perrot C, Feltz P, Poulter MO. Recombinant GABAA receptor desensitization: the role of the gamma 2 subunit and its physiological significance. *J Physiol* 1996;497 :145–59. [PubMed: 8951718]Pt 1
16. Dudeck O, Lubben S, Eipper S, Knorle R, Kirsch M, Honegger J, Zentner J, Feuerstein TJ. Evidence for strychnine-sensitive glycine receptors in human amygdala. *Naunyn Schmiedebergs Arch Pharmacol* 2003;368:181–7. [PubMed: 12923611]
17. Ernst M, Bolla K, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, Cadet JL, Kimes AS, London ED. Decision-making in a risk-taking task: a PET study. *Neuropsychopharmacology* 2002;26:682–91. [PubMed: 11927193]
18. Fanselow MS, LeDoux JE. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* 1999;23:229–32. [PubMed: 10399930]
19. Farroni JS, McCool BA. Extrinsic factors regulate partial agonist efficacy of strychnine-sensitive glycine receptors. *BMC Pharmacol* 2004;4:16. [PubMed: 15301692]
20. Galvan A, Hare TA, Davidson M, Spicer J, Glover G, Casey BJ. The role of ventral frontostriatal circuitry in reward-based learning in humans. *J Neurosci* 2005;25:8650–6. [PubMed: 16177032]

21. Grenningloh G, Pribilla I, Prior P, Multhaup G, Beyreuther K, Taleb O, Betz H. Cloning and expression of the 58 kd beta subunit of the inhibitory glycine receptor. *Neuron* 1990;4:963–70. [PubMed: 2163264]
22. Grenningloh G, Rienitz A, Schmitt B, Methfessel C, Zensen M, Beyreuther K, Gundelfinger ED, Betz H. The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature* 1987;328:215–20. [PubMed: 3037383]
23. Griebel G, Belzung C, Perrault G, Sanger DJ. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology (Berl)* 2000;148:164–170. [PubMed: 10663431]
24. Handley SL, McBlane JW, Critchley MA, Njung'e K. Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. *Behav Brain Res* 1993;58:203–10. [PubMed: 8136047]
25. Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 1987;325:529–31. [PubMed: 2433595]
26. Killcross S, Robbins TW, Everitt BJ. Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature* 1997;388:377–80. [PubMed: 9237754]
27. Kumar R, Palit G, Singh JR, Dhawan BN. Comparative behavioural effects of benzodiazepine and non-benzodiazepine anxiolytics in rhesus monkeys. *Pharmacol Res* 1999;39:437–44. [PubMed: 10373237]
28. Masaoka Y, Hirasawa K, Yamane F, Hori T, Homma I. Effects of left amygdala lesions on respiration, skin conductance, heart rate, anxiety, and activity of the right amygdala during anticipation of negative stimulus. *Behav Modif* 2003;27:607–19. [PubMed: 14531157]
29. McCool BA, Botting SK. Characterization of strychnine-sensitive glycine receptors in acutely isolated adult rat basolateral amygdala neurons. *Brain Res* 2000;859:341–51. [PubMed: 10719083]
30. McCool BA, Farroni JS. Subunit composition of strychnine-sensitive glycine receptors expressed by adult rat basolateral amygdala neurons. *Eur J Neurosci* 2001;14:1082–1090. [PubMed: 11683900]
31. McCool BA, Frye GD, Pulido MD, Botting SK. Effects of chronic ethanol consumption on rat GABA (A) and strychnine-sensitive glycine receptors expressed by lateral/basolateral amygdala neurons. *Brain Res* 2003;963:165–77. [PubMed: 12560122]
32. McNamara D, Dingledine R. Dual effect of glycine on NMDA-induced neurotoxicity in rat cortical cultures. *J Neurosci* 1990;10:3970–6. [PubMed: 1980134]
33. Moller C, Sommer W, Thorsell A, Heilig M. Anxiogenic-like action of galanin after intra-amygdala administration in the rat. *Neuropsychopharmacology* 1999;21:507–12. [PubMed: 10481834]
34. Mori M, Gahwiler BH, Gerber U. Beta-alanine and taurine as endogenous agonists at glycine receptors in rat hippocampus in vitro. *J Physiol* 2002;539:191–200. [PubMed: 11850512]
35. Motulsky, H. *Intuitive biostatistics*. xx. New York: Oxford University Press; 1995. p. 386
36. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. London: Academic Press; 1997. p. 80
37. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 1985;14:149–67. [PubMed: 2864480]
38. Prasad A, Imamura M, Prasad C. Dehydroepiandrosterone decreases behavioral despair in high- but not low-anxiety rats. *Physiol Behav* 1997;62:1053–7. [PubMed: 9333199]
39. Pribilla I, Takagi T, Langosch D, Bormann J, Betz H. The atypical M2 segment of the beta subunit confers picrotoxinin resistance to inhibitory glycine receptor channels. *Embo J* 1992;11:4305–11. [PubMed: 1385113]
40. Ramos A, Mormede P. Stress and emotionality: a multidimensional and genetic approach. *Neurosci Biobehav Rev* 1998;22:33–57. [PubMed: 9491939]
41. Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev* 1997;21:801–10. [PubMed: 9415905]
42. Samson HH, Chappell A. Muscimol injected into the medial prefrontal cortex of the rat alters ethanol self-administration. *Physiol Behav* 2001;74:581–7. [PubMed: 11790418]

43. Sananes CB, Davis M. N-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behav Neurosci* 1992;106:72–80. [PubMed: 1554439]
44. Sanders SK, Shekhar A. Regulation of anxiety by GABAA receptors in the rat amygdala. *Pharmacol Biochem Behav* 1995;52:701–6. [PubMed: 8587908]
45. Schmieden V, Kuhse J, Betz H. Agonist pharmacology of neonatal and adult glycine receptor alpha subunits: identification of amino acid residues involved in taurine activation. *Embo J* 1992;11:2025–32. [PubMed: 1376243]
46. See RE, McLaughlin J, Fuchs RA. Muscarinic receptor antagonism in the basolateral amygdala blocks acquisition of cocaine-stimulus association in a model of relapse to cocaine-seeking behavior in rats. *Neuroscience* 2003;117:477–83. [PubMed: 12614687]
47. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965:591–611.
48. Valeyev AY, Hackman JC, Holohean AM, Wood PM, Katz JL, Davidoff RA. GABA-Induced Cl⁻ current in cultured embryonic human dorsal root ganglion neurons. *J Neurophysiol* 1999;82:1–9. [PubMed: 10400929]
49. Vandenberg RJ, French CR, Barry PH, Shine J, Schofield PR. Antagonism of ligand-gated ion channel receptors: two domains of the glycine receptor alpha subunit form the strychnine-binding site. *Proc Natl Acad Sci U S A* 1992;89:1765–9. [PubMed: 1311851]
50. Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. *Neurosci Biobehav Rev* 2001;25:275–86. [PubMed: 11378181]
51. Zangrossi H, Graeff FG. Behavioral effects of intra-amygdala injections of GABA and 5-HT acting drugs in the elevated plus-maze. *Braz J Med Biol Res* 1994;27:2453–6. [PubMed: 7640637]

Figure 1. Cannula placement was consistent with injections into the lateral/basolateral amygdala. Points (●) represent the apparent tips of guide cannulae revealed during post-hoc anatomical analysis of individual animals (see text). Numbers at right indicate the anterior-to-posterior position of each slice relative to the Bregma (from [36]). Note the majority of guide cannulae appeared to terminate the anterior portions of the lateral nucleus.

Figure 2.

Anxiety-like behavior in aCSF-injected animals ($n=45$) can be segregated into different behavioral responses to the elevated plus maze and the light/dark box. **(A)** Open-arm time distribution of all aCSF-injected animals tested in the elevated plus maze. The data were divided into bins and plotted according to the number of animals in each bin. The entire distribution failed the Shapiro-Wilk test for normality (see Methods); a forced fit to a single Gaussian distribution (dashed line) yielded a mean of $31.1 \pm 51.0\%$ of the population mean and an area of 1470 ± 700 . Solid lines represent Gaussian fits of the binned data separated into two groups: 'low open-arm time' animals (left) with a mean = $37.4 \pm 5.0\%$ and area = 695.2 ± 98.5 ; and, 'high open-arm time' animals (right) with a mean = $117.7 \pm 23.4\%$ sec and area = 648 ± 225 . **(B)**

Light-side time distribution of all aCSF-injected animals tested in the light/dark box. The dashed line represents a Gaussian fit across the entire population with a mean= $125.6 \pm 13.9\%$ and area= 2174 ± 293 . Solid lines represent Gaussian fits for 'low light-side time' animals (left; mean= $73.1 \pm 7.0\%$, area= 1303 ± 172) and 'high light-side time' animals (right; mean= $171.3 \pm 4.8\%$, area= 1229 ± 159).

Figure 3.

In the elevated plus maze, strychnine selectively reduces anxiety-like behavior in 'low open-arm time' animals. Animals were segregated into those spending < or ≥ 90sec in open arms following aCSF injection into the basolateral amygdala (see Fig. 2). Values are expressed as percent of the population mean for aCSF-injected animals. **(A)** Strychnine injection (0.5pmol, ■) significantly increased open-arm time in 'low open-arm time' animals relative to aCSF but did not affect this variable in 'high open-arm time' individuals. There was a trend for an interaction between treatment and phenotype ($P \sim 0.07$, repeated measures two-way ANOVA with phenotype and strychnine as main factors) and a significant main effect of phenotype (§§§, $P < 0.001$) with post-hoc analysis indicating a significant effect of strychnine in the 'low open-arm time' animals (*- $P < 0.05$, Bonferroni posttest). **(B)** Strychnine decreased closed-arm time in 'low open-arm time' but not in 'high open-arm time' individuals. Statistical analysis revealed a significant interaction between strychnine injection and phenotype ($P < 0.05$) with a

significant decrease in closed-arm times in 'low open-arm time' animals (**- $P < 0.01$). **(C)** Strychnine did not influence the amount of time animals spent at the junction between open and closed arms regardless of phenotype. **(D)** Strychnine did not influence the number of open-arm entries. However, there was a significant main effect of phenotype with 'low open-arm time' animals making fewer open-arm entries (§§, $P < 0.01$, repeated measures two-way ANOVA).

Figure 4.

Injection of strychnine into the lateral/basolateral amygdala modulates anxiety-like behavior in the light/dark box. **(A)** There was a significant interaction between phenotype and strychnine microinjection relative to aCSF ($P < 0.01$, two-way repeated measures ANOVA) with strychnine (■) significantly increasing the total amount time 'low light-side time' animals spent in this side of the apparatus (left; ** $-P < 0.01$, Bonferroni posttest). Strychnine microinjection did not influence this variable in 'high light-side time' individuals (right). **(B)** Strychnine significantly increased the number of transitions between light and dark compartments in the 'high light-side time' animals ($P < 0.05$ with Bonferroni's posttest) but not the 'low light-side time' individuals. The analysis also indicated a significant main-effect of phenotype (§§, $P < 0.01$, repeated measures two-way ANOVA). **(C)** Strychnine significantly reduced re-entry latency (the amount of time an animal takes to re-enter the light-side of the apparatus following its initial egress to the dark-side) in 'low light-side time' individuals (left; * $-P < 0.05$,

Bonferroni posttest) but not the other phenotype (right). **(D)** Strychnine did not alter the total distance traveled during the test for either phenotype, suggesting the drug's effects on other parameters were not significantly influenced by locomotor-related behaviors. However, 'high light-side time' individuals traveled a greater distance than 'low light-side time' animals ($P < 0.01$ for phenotype).

Figure 5.

In the elevated plus maze, taurine disrupts anxiety-like behavior in 'low open-arm time' animals. (A) Times spent (expressed as percent of population mean for aCSF-injected animals) in the open arms for 'low open-arm time' (left) and 'high open-arm time' (right) animals injected with aCSF (□), 5pmol (0.6ng) taurine (▤), 50pmol (6.3ng) taurine (▨), and 1nmol (125ng) taurine (■). There was a significant main-effect of phenotype (§§- $P < 0.01$, repeated measures two-way ANOVA). More importantly, the 50pmol taurine dose increased open-arm time relative to aCSF in 'low open-arm time' animals (*-, $P < 0.05$, Bonferoni post-test). (B & C) Neither closed-arm time (B) nor junction time (C) were significantly effected by taurine microinjection, regardless of animal phenotype. However, there was a significant main-effect of phenotype for closed-arm time (B; §- $P < 0.05$, two-way ANOVA). (D) For the number of open-arm entries, there was a significant main-effect of dose ($P < 0.01$, two-way ANOVA).

However, this variable was un-affected in 'high open-arm time' animals but was significantly increased at the 50pmol (6.3ng) dose in 'low open-arm time' individuals (**- $P < 0.01$ relative to aCSF; Bonferroni post-test). There was no significant difference between total numbers of arm entries in the aCSF-injected animals from the different phenotypes (not shown).

Figure 6.

Taurine injection into the lateral/basolateral amygdala has negligible effects on anxiety-like behavior expressed in the light/dark box. Taurine did not produce significant dose-dependent effects on light-side time (**A**), the number of light/dark transitions (**B**), re-entry latency (**C**), or the amount of distanced animals moved during the assay (**D**).

Figure 7.

Populations defined by light-side time in the light/dark box are differentially sensitive amygdala strychnine and taurine microinjection when measured in the plus maze. (A) 'Low light-side time' and 'high light-side time' animals were identified from the aCSF-related behaviors expressed in the light/dark box (see Fig. 2). When behavior of these populations were examined in the plus maze following aCSF or strychnine microinjection, aCSF-injected 'low light-side time' individuals spent significant less time in the open-arms of the plus maze compared to aCSF-injected animals in the 'high light-side time' population. There was a significant interaction between strychnine and phenotype ($P < 0.01$, two-way repeated measures ANOVA) with the drug significantly increasing open-arm time in 'low light-side time', but

not 'high light-side time', animals (**- $P < 0.01$ with Bonferroni's post test). Significant effects of strychnine were also demonstrated for closed arm time (see text) but not for open arm entries (**B**) or junction time. (**C**) Taurine differentially affects the plus maze behavior of 'low light-side time' and 'high light-side time' populations. Along with a significant interaction between phenotype and taurine ($P < 0.05$, two-way ANOVA), the 1nmol (125ng) taurine dose significantly increased open-arm time in the 'low light-side time' individuals (*- $P < 0.05$, Bonferroni post-test) but not in 'high light-side time' animals. Other plus maze dependent variables, like open arm entries (**D**), closed-arm time, and junction-time, were not significantly altered by taurine microinjection in either population of animals.

Table 1

Summary of Strychnine Effects on Anxiety-Like Behavior

	aCSF ^a	Strychnine ^a
Plus Maze		
Open-Arm Time (sec)	69.7±13.7	91.2±13.7 ^b
Closed-Arm Time (sec)	107.2±14.1	83.5±12.1 [*]
No. Arm Entries	13.2±1.2	12.5±1.0
Open-Arm Entries (% Total)	56.9±6.6	62.3±6.7
Light/Dark Box		
Time in Light (sec)	101.1±16.0	116.7±12.5 ^b
Side Transitions (#)	5.6±0.6	7.3±0.8 [*]
Re-entry Latency (sec)	70.5±17.6	40.7±14.2 [*]
Move Distance (cm)	1477±40	1552±41 ^b

^a 0.5µl aCSF or 0.5pmol (0.17ng) strychnine injected bilaterally, n = 20 (2 cohorts)

^b paired t-test, P<0.1;

^{*} paired t-test, P<0.05

Table 2

Summary of Taurine Effects on Anxiety-Like Behavior

	aCSF ^a	5pmol (0.6ng) Taurine	50pmol (6.3ng) Taurine ^a	1nmol (125ng) Taurine ^a
Plus Maze^b				
Open-Arm (sec)	74.0±15.1	80.6±17.7	91.1±10.2	84.4±11.8
Closed-Arm (sec)	120.8 ±15.4	146.6±15.7	115.7±13.4	104.5±14.7
No. Arm Entries	12.7±1.3	13.4±1.6	14.3±1.5	14.0±1.7
Open-Arm Entries (%)	62.9±5.7	53.0±8.5	78.2±4.4	67.7±6.9
Light/Dark Box^b				
Time in Light (sec)	142.8 ±19.3	152.2±16.5	132.2±19.3	113.0±22.7
Side Transitions (#)	6.3±0.7	7.9±0.9	6.4±0.8	6.6±1.1
Re-entry Latency (sec)	42.2±19.6	39.8±21.7	44.9±24.3	76.4±31.5
Move Distance (cm)	1261±43	1194±52	1384±61	1379±57

^a 0.5µl injected bilaterally^b Experiment 1, n=12 (aCSF, 50pmol, and 1nmol taurine); Experiment 2, n=13 (aCSF and 5pmol taurine). aCSF-values did not differ between experiments and were combined for this analysis.