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REPORTS OF ORIGINAL INVESTIGATIONS

# Evaluation of a novel mouse model of intracisternal strychnine-induced trigeminal allodynia

# Évaluation d'un modèle de souris innovant de l'allodynie du trijumeau induite par la strychnine administrée par voie intracisternale

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**Abstract**

**Purpose** *Intractable neuropathic dynamic allodynia remains one of the major symptoms of human trigeminal neuropathy and is commonly accepted to be the most excruciatingly painful condition known to humankind. At present, a validated animal model of this disorder is necessary for efficient and effective development of novel drug treatments. Intracisternal strychnine in rats has been shown to result in localized trigeminal dynamic allodynia, thus representing a possible model of trigeminal neuralgia. The purpose of this study was to validate a mouse model of trigeminal glycinergic inhibitory dysfunction using established positive (carbamazepine epoxide) and negative (morphine) controls.*

**Methods** *The actions of conventional first-line treatment (carbamazepine epoxide [CBZe]) and clinically ineffective morphine were tested for trigeminal dynamic mechanical allodynia produced by intracisternal strychnine. In mice under halothane anesthesia, we injected either strychnine (0.3 μg), strychnine with CBZe (4 ng), or artificial cerebrospinal fluid (aCSF) intracisternally (i.c.). In a separate set of experiments, subcutaneous morphine (3 mg·kg$^{-1}$ sc) was injected with intracisternal strychnine. Dynamic mechanical allodynia was induced by stroking the fur with polyethylene (PE-10) tubing. The response of each mouse was rated to determine its allodynia score, and scores of each group were compared. In addition, in a separate dichotomous disequilibrium study, pairs of mice were injected with strychnine/saline, strychnine/strychnine-CBZe, or strychnine/strychnine-morphine. A blinded observer recorded which mouse of each pair had the greater global pain behaviour.*

**Results** *Strychnine (i.c.) produced higher quantitative allodynia scores in the trigeminal distribution (mean 81.5%; 95% confidence interval [CI] 76.4 to 86.6) vs the aCSF group (mean 11.3%; 95% CI 8.1 to 14.4) ($P < 0.0001$). Carbamazepine epoxide (i.c.) completely abolished allodynia when co-injected with strychnine (mean 83.2%; 95% CI 78.1 to 88.4) vs strychnine alone (mean 3.2%; 95% CI −0.9 to 7.2) ($P < 0.0001$). Morphine co-injected with strychnine did not result in reduced allodynia (mean 65.7%; 95% CI 42.0 to 89.4) compared with strychnine alone (mean 87.6%; 95% CI 77.6 to 97.6) ($P = 0.16$). In a further global allodynia assessment, strychnine (i.c.) produced greater allodynia than both aCSF and strychnine administered with CBZe ($P = 0.03$). Morphine (ip) administered with strychnine did not result in reduced global allodynia compared with strychnine administered alone ($P = 1.0$).*

**Author contributions** *Il-Ok Lee* performed the experiments. *Il-Ok Lee, Craig R. Ries, Stephan K.W. Schwarz, Ernest Puil,* and *Bernard A. MacLeod* contributed to the experimental design and the rationale for the experiments. *Ryan A. Whitehead* contributed to data analysis and the writing of the manuscript. *Craig R. Ries, Stephan K.W. Schwarz,* and *Ernest Puil* edited the manuscript. *Bernard A. MacLeod* initiated the project and participated in the experimental techniques and in the preparation and revision of the manuscript.

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**Conclusion** *In this study, we have developed and validated a novel murine model of trigeminal dynamic allodynia induced by intracisternal strychnine. The use of mice to study trigeminal allodynia has many benefits, including access to a vast repository of transgenic mouse variants, ease of handling, low cost, and minimal variance of results. The present model may have utility in screening drug treatments for dynamic mechanical allodynia resulting from trigeminal neuropathies.*

**Résumé**

**Objectif** *L'allodynie dynamique neuropathique réfractaire demeure l'un des symptômes majeurs de la neuropathie du trijumeau chez l'humain. On la considère généralement comme l'état le plus insupportablement douloureux pour l'humain. À l'heure actuelle, un modèle animal validé de ce trouble est nécessaire pour mettre au point de façon efficiente et efficace de nouveaux traitements médicamenteux. Il a été démontré que la strychnine administrée par voie intracisternale chez le rat entraînait une allodynie dynamique localisée du trijumeau, ce qui constituerait un modèle possible de névralgie faciale. L'objectif de cette étude était de valider un modèle murin du dysfonctionnement de l'inhibition glycinergique du trijumeau en se servant de témoins positifs (époxyde de carbamazépine) et négatifs (morphine) établis.*

**Méthode** *Les effets du traitement traditionnel de première intention (époxyde de carbamazépine [CBZe]) et de la morphine, inefficace d'un point de vue clinique, ont été testés pour traiter une allodynie mécanique dynamique du trijumeau provoquée par de la strychnine administrée par voie intracisternale. Nous avons injecté de la strychnine (0,3 µg), de la strychnine avec du CBZe (4 ng) ou un liquide céphalorachidien artificiel (aCSF) par voie intracisternalle (i.c.) chez des souris anesthésiées avec de l'halothane. Dans d'autres tests, de la morphine sous-cutanée (3 mg·kg$^{-1}$ sc) a été injectée avec la strychnine intracisternale. L'allodynie mécanique dynamique a été induite en caressant le pelage de l'animal avec un tube de polyéthylène (PE-10). La réaction de chaque souris a été évaluée afin de déterminer son score d'allodynie, et les scores de chaque groupe ont été comparés. De plus, dans une étude de déséquilibre dichotomique distincte, on a injecté les produits suivants chez des souris appariées : strychnine/solution salée, strychnine/strychnine-CBZe, ou strychnine/strychnine-morphine. Un observateur en aveugle a enregistré quelle souris, dans chaque paire, montrait le comportement douloureux global le plus important.*

**Résultats** *La strychnine (i.c.) a entraîné des scores d'allodynie quantitative plus élevée dans les distributions nerveuses du trijumeau (moyenne 81,5 %; intervalle de confiance [IC] 95 % 76,4 à 86,6) que dans le groupe aCSF (moyenne 11,3 %; IC 95 % 8,1 à 14,4) ($P < 0,0001$). L'époxyde de carbamazépine (i.c.) a complètement aboli l'allodynie lorsqu'elle était injectée conjointement à de la strychnine (moyenne 83,2 %; IC 95 % 78,1 à 88,4) par rapport à la strychnine administrée seule (moyenne 3,2 %; IC 95 % −0,9 à 7,2) ($P < 0,0001$). La morphine injectée conjointement à la strychnine n'a pas provoqué de réduction de l'allodynie (moyenne 65,7 %; IC 95 % 42,0 à 89,4) par rapport à la strychnine seule (moyenne 87,6 %; IC 95 % 77,6 à 97,6) ($P = 0,16$). Dans une évaluation globale de l'allodynie plus approfondie, la strychnine (i.c.) a produit une allodynie plus importante que l'aCSF et la strychnine administrée avec de la CBZe ($P = 0,03$). La morphine (ip) administrée avec la strychnine n'a pas provoqué de réduction de l'allodynie globale par rapport à la strychnine ($P = 1,0$).*

**Conclusion** *Dans cette étude, nous avons mis au point et validé un modèle murin innovant d'allodynie dynamique du trijumeau induite par la strychnine intracisternale. L'utilisation de souris pour étudier l'allodynie du trijumeau comporte de nombreux avantages, notamment l'accès à un vaste répertoire de variantes de souris transgéniques, une facilité de maniement, un faible coût et une variance minime des résultats. Le modèle présenté ici pourrait être utile pour faire une sélection préliminaire des traitements médicamenteux de l'allodynie mécanique dynamique causée par des neuropathies du trijumeau.*

There is a pressing need for effective therapies for intractable dynamic mechanical allodynia in conditions such as trigeminal neuralgia.[1] Indeed, trigeminal neuralgia is widely considered to be the most excruciatingly painful condition in existence for humans. The symptoms consist of remitting and relapsing stabbing pain in facial regions in response to light touch, mediated by aberrant local trigeminal nerve function.[2] Studies of trigeminal neuralgia drug treatments in humans are difficult to implement due to the episodic and variable nature of the disorder.[3] There is therefore a need for accurate, predictive, and cost-effective animal models for development of novel drug candidates. Recently, trigeminal glycinergic inhibitory dysfunction has been shown in rats to produce localized dynamic allodynia, a central feature of human trigeminal neuralgia.[4,5] While electrophysiological, behavioural, and pharmacological studies *in vivo* have shown that this produces many of the features of clinical trigeminal neuropathies, the model requires validation for clinically effective agents.[6] Nonetheless, the clinically ineffective agent, morphine, has also been shown to be ineffective in reversing rat dynamic mechanical allodynia resulting from strychnine-induced allodynia. This suggests a possible relationship with the human disorder.[4,5] In this study, we developed and evaluated

an alternative mouse preparation of intracisternal (i.c.) strychnine-based trigeminal neuropathic pain and examined the effects of artificial cerebrospinal fluid (aCSF), morphine, and carbamazepine in order to assess predictive efficacy for screening trigeminal neuralgia drug candidates. The evaluation and development of a murine preparation analogous to the rat model of trigeminal glycinergic inhibitory dysfunction takes advantage of the availability of genetic mouse variants, resulting in lower costs for animals and animal housing and reduced drug dose requirements. Studies of the comparative efficacy of clinically effective and ineffective agents are needed for trigeminal strychnine-induced glycinergic inhibitory dysfunction.[7] In the present experiments, we have validated our novel mouse model of human trigeminal neuralgia by studying the effects of the first-line pharmacological agent, carbamazepine, along with those of morphine, which is clinically ineffective.

## Methods

### Animals

Adult female CD-1 mice weighing 20-25 g were housed in an approved facility with a 12-hr light/dark cycle and free access to food and tap water. The experiments were conducted in accordance with the Guidelines of the Canadian Council on Animal Care and were approved by The University of British Columbia Animal Care Committee.

### Prior experiments

Intracisternal injections were conducted such that needle placement was directed into the cleft between the occiput and atlas vertebra through intact skin. The needle tip was inserted into the cleft such that near-vertical position was maintained, and it was then rotated forwards such that the bent portion was kept in close contact with the internal surface of the occiput for the entire length. After injection of 5 μL of drug solution, the syringe was held in the same position at an angle of 45-55° for five seconds to minimize fluid outflow. In a set of prior experiments, the accuracy of the injection method was tested using 1% methylene blue solution, allowing for assessment of dye distribution in the intracisternal space. Dye was distributed in the cisterna magna up to the occipital surface of the brain in 98% of mice tested. In pilot experiments, we investigated the effects of i.c. glycine on strychnine-induced allodynia. A (5 μL) solution of 200 μM (0.3344 μg) of strychnine in aCSF (composition below) was co-injected with 0, 20, 200, or 400 μM of glycine into the cisterna magna.

### Intracisternal injections

Mice were anesthetized with a halothane/air mixture in a 500 mL induction chamber. A rubber mask was used to maintain halothane anesthesia and to immobilize the head. Halothane was administered through a Mapleson D circuit with a Dräger vaporizer (Draeger Medical Inc., Telford, PA, USA). To maximize the opening to the cisterna magna, the neck was flexed over a modelling clay form, and the space between the occiput and C1 segment was identified by palpation with the index finger (Lee *et al.*, 2011).[8] A 10 μL microvolume precision syringe was used (Hamilton, Reno, NV, USA) with a 26G needle that had a non-coring bevelled tip partially covered by polyethylene (PE-10) tubing to limit penetration to 4 mm. Dynamic allodynia was restricted to the trigeminal distribution. No morbidity was observed (such as piloerection, immobility, decreased respiratory rate, or decreased responsiveness to touch).

### Drug administration

Three separate experiments were performed with six pairs of mice in each experiment for a total of 36 mice. Each animal was used only once. Experiments were conducted from 09:00 to 16:00 in a quiet room. All experiments were recorded by video camera (Canon, Tokyo, Japan) and saved to a computer file. Drug dosage reported here is the molarity in total volume of injected solution (5 μL). Even after sonication of carbamazepine for $> 20$ min, carbamazepine did not result in a homogeneous emulsion. We therefore used the water-soluble active carbamazepine metabolite, carbamazepine epoxide (CBZe; Sigma Chemical, St. Louis, MO, USA), which has comparable pharmacological properties.[9] The maximal soluble dose of CBZe (3.2 μM) had no obvious systemic side effects, as indicated by an absence of piloerection, hypothermia, and sedation. Strychnine and CBZe were dissolved in aCSF containing (in mM): 124 NaCl, 26 $NaHCO_3$, 1.25 $NaH_2PO_4$, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, and 10 dextrose at pH = 7.3-7.4. Strychnine and CBZe were injected intracisternally. Morphine HCl (Reckitt & Colman, Toronto, ON, Canada) was dissolved in saline (3 $mg \cdot kg^{-1}$) and injected subcutaneously at 5 $mL \cdot kg^{-1}$ body weight. All drug combinations were injected ten minutes prior to recordings.

### Experimental design

Injections were prepared and coded by an investigator independent from the experimenter, allowing for blinding to group allocation. One mouse of each of six pairs in each experiment received one injection for each comparison. Mice were placed on opposite sides of a transparent plastic

cage (30 cm long, 15 cm high, and 15 cm wide) divided by an opaque internal wall. The maximum time variation between control and drug injections was 30 sec. Randomization of drug allocation was determined by generating a random sequence using an Internet-based computer randomizer that generates randomization as a function of atmospheric noise (www.random.org; Trinity College, Dublin). The solutions were prepared by a scientist independent from the experimenter and blinded using a second order notation system for the said scientist's post experiment retrieval. Recovery from anesthesia occurred less than four minutes after discontinuation of halothane. Assessments were made at four, six, eight, ten, 12, and 14 min following recovery from anesthesia.

### Allodynia testing

We tested for dynamic mechanical allodynia by observing the response to lightly brushing an 8-cm long PE-10 tube against the grain of the animal's fur. Flinching, scratching, and/or agitation were indicative of a dynamic mechanical allodynia. The brushing was applied first to the face and then to the neck, followed by the back, forelimbs, and hind limbs. There were no allodynic responses in the back, forelimbs, and hind limbs, areas that are not in the anatomical distribution of the trigeminal nerve. To determine if a tested drug produced a generalized analgesia, we observed tail-flick responses to a curved 50-mm vascular tail clamp (Bulldog type Serrefine; International Fine Science Tools Inc., North Vancouver, BC, Canada). An independent observer reviewed each experiment through a videotaped record. The absence of response to stimulus within the distribution of the trigeminal nerve was scored as 0; eye squinting or backward folding of ears as 1; head withdrawal as 2; and face scratching as 3.[10] In treatment groups, we determined each individual animal's allodynia score as the sum of the scores at each of the six two-minute intervals. The results are presented as percent of maximum allodynia with mean (standard deviation) for each of the treatment groups. The experimenter's global impression of the relative allodynia in each pair of mice was used to substantiate these allodynia score determinations.

### Statistical analysis

The allodynia score data for each of the two drug groups in the individual experiments were analyzed using Student's *t* test. For each of the experiments, the allodynia scores were compared between drug groups using Student's two-sample *t* test for each of the two drug groups in the individual experiments. Allodynia score data were analyzed using Student's *t* test using SigmaStat®, v. 3.01 (Systat Software Inc.; Chicago, IL, USA). In previous comparisons between intracisternal strychnine and aCSF, mean scores (standard deviation) were 3.2 (0.2) *vs* 0.5 (0.1) in five mice ($P < 0.001$) (Miracourt *et al.*, 2007). As the anticipated effect size of isovaline was > 90% for a power 0.8, the sample size was six to eight mice. The global impression data were analyzed using the binomial test under the null hypothesis of no difference (i.e., $\pi = 0.5$).

## Results

### Effects of intracisternal strychnine compared with aCSF control

In the preliminary study, 200 μM intracisternal strychnine, the largest dose that produced allodynia without convulsions or agitation, was chosen as the glycine inhibitory dose. Allodynia lasted from the time of emergence from anesthesia to more than 20 min after strychnine injection. Strychnine (i.c.) produced higher quantitative allodynia scores in the trigeminal distribution (mean 81.5%; 95% confidence interval [CI] 76.4 to 86.6) *vs* the aCSF group (mean 11.3%; 95% CI 8.1 to 14.4) ($P < 0.0001$). Neither strychnine (200 μM) nor aCSF produced motor abnormalities or convulsions. Between stimuli, animals appeared behaviourally normal and did not exhibit excessive grooming, piloerection, or abnormal movement. None of the six animals injected with aCSF showed allodynia over the entire test period, but three showed transient initial allodynia lasting less than four minutes. Strychnine produced greater comparative allodynia in six of six pairs of mice over all time periods compared with those injected with aCSF alone (Table).

### Effects of glycine on strychnine-induced allodynia

Neither glycine 20 μM ($n = 1$) nor 200 μM, nor 400 μM ($n = 3$) co-injected i.c. with strychnine changed strychnine-induced allodynia ($n = 6$). Nevertheless, the highest dose of glycine (400 μM) produced respiratory depression and death in three of three animals, resulting in discontinuation of these experiments.

### Effects of CBZe on strychnine-induced allodynia

In the absence of strychnine, CBZe (4 ng intracisternally) produced no apparent sensory or motor deficits. The dose was chosen based on the relative potency of CBZe to carbamazepine. A normal tail-flick response to a vascular clamp was present at each measurement time. Carbamazepine epoxide (i.c.) completely abolished allodynia when co-injected with strychnine (Fig. 1A, B) (mean 83.2%; 95% CI 78.1 to 88.4) *vs* strychnine alone (mean 3.2%; 95% CI −0.9 to 7.2) ($P < 0.0001$). All six mice

co-injected with CBZe and strychnine showed markedly reduced allodynia scores over all time periods, as seen in Fig. 1A and in their cumulative scores (Fig. 1B). In six of six pairwise comparisons, less allodynia ensued in animals receiving carbamazepine epoxide than in those injected with strychnine alone ($P = 0.03$, Table).

### Effects of subcutaneous morphine on strychnine-induced allodynia

Morphine co-injected with strychnine did not result in reduced allodynia (mean 65.7%; 95% CI 42.0 to 89.4) compared with strychnine alone (Fig 2A, B) (mean 87.6%; 95% CI 77.6 to 97.6) ($P = 0.16$). Three of six mice co-injected with CBZe and morphine showed reduced allodynia scores over all time periods, and three showed increased allodynia scores in pairwise comparisons ($P = 1.0$) (Table).

## Discussion

In this study, CBZe selectively decreased intracisternal trigeminal mechanical allodynia induced by glycinergic inhibitory dysfunction in mice; but morphine did not result in a reduction in allodynia. These results validate this preparation as a mouse model of human trigeminal mechanical allodynia. The similarities of intracisternal strychnine-induced glycine disinhibition to clinical trigeminal neuropathy include the presence of dynamic allodynia[1] and its restriction to the distribution of the trigeminal nerve distribution.[4] Strychnine did not produce changes in sensorimotor activity in areas other than the trigeminal distribution, allowing for assessment of nervous system dysfunction specific to the trigeminal nerve.

Models of trigeminal allodynia, such as infraorbital nerve constriction,[10] produce secondary changes in the central nervous system resulting from peripheral nerve damage, while intracisternal strychnine does not produce peripheral nerve destruction and therefore allows for reproducible assessment of trigeminal nerve dysfunction.

In the treatment of trigeminal neuropathy in humans, the water-soluble active carbamazepine metabolite, carbamazepine-10, 11-epoxide, has similar or greater potency than its parent drug, carbamazepine, and contributes to its antineuralgic effects.[9] Systemic administration of carbamazepine is widely documented and would be expected to achieve similar brainstem therapeutic target levels; however, systemic administration could complicate behavioural observations as there are several active metabolites of carbamazepine with differing potencies and side effects. While the efficacy of CBZe is higher in our model than that of carbamazepine in human trigeminal neuralgia, the ability to manifest and localize the effect was considered to be of greater importance than establishing analogous dosing potencies.

Morphine is presumed to accentuate allodynia via several excitatory mechanisms; however, the lack of effectiveness of intracisternal morphine on dynamic allodynia, as commonly occurs in clinical trigeminal neuralgia, was not attributable to opioid receptor actions.[11] Subcutaneous morphine (at $\leq 3$ mg·kg$^{-1}$) has been shown to block peripheral mechanical allodynia without sedation, allowing us to use morphine as a control for the presence of non-specific peripheral mechanical allodynia.[5,10,12] To examine the

**Table** Relative allodynia for pairs of mice with differing treatments

| Relative Strychnine (Str)-induced Allodynia in Mouse Pairs | | |
|---|---|---|
| Paired condition | Mouse with greater allodynia | *P* value |
| Str *vs* aCSF | Str in 6 of 6 pairs | $P = 0.03$ |
| Str *vs* Str + CBZe | Str in 6 of 6 pairs | $P = 0.03$ |
| Str *vs* Str + morphine (3 mg·kg$^{-1}$ *sc*) | Str in 3 of 6 pairs<br>morphine in 3 of 6 pairs | $P = 1.0$ |
| Str *vs* Str + isovaline 5 µL l of 4 µM (3 µgm)[18] | Str in 8 of 8 pairs[18] | $P = 0.008$ |

Three groups of six pairs assessed for significance using the binomial test. One group of eight pairs (isovaline) is from previously published data.[18] CBZe = carbamazepine epoxide; aCSF = artificial cerebrospinal fluid

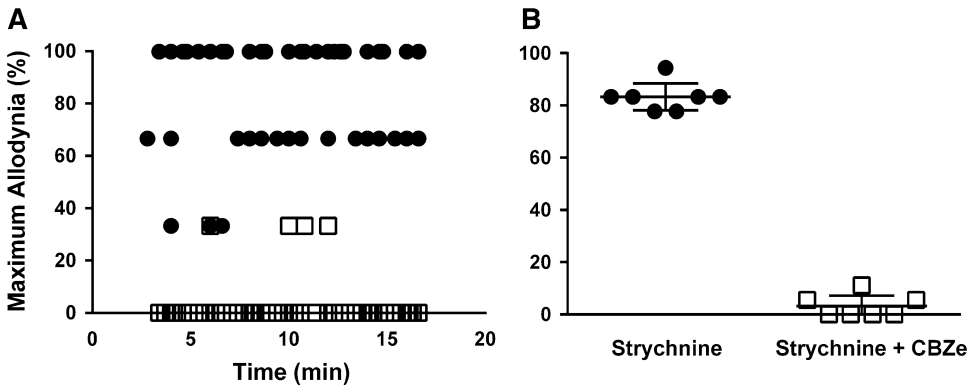


**Fig. 1** Effects of carbamazepine epoxide on strychnine-induced allodynia. A) Allodynia scores for each observation period (strychnine, filled circles; strychnine + carbamazepine epoxide [CBZe], open squares). B) Cumulative allodynia scores from four to 14 min normalized to maximum possible effect (bars represent mean, 95% confidence interval; $n = 6$; two-tailed Student's *t* test, $P < 0.0001$)

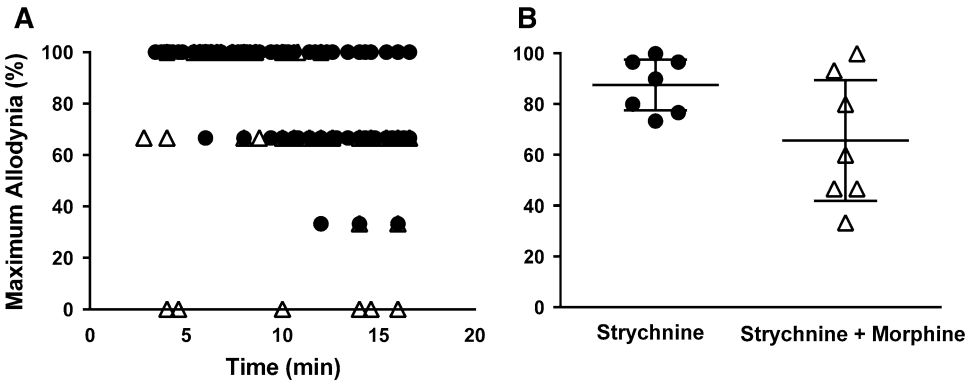


**Fig. 2** Effects of morphine on strychnine-induced allodynia. A) Allodynia scores for each observation period (strychnine, filled circles; strychnine + morphine, open squares). B) Cumulative allodynia scores from four to 14 min normalized to maximum possible effect (bars represent mean, 95% confidence interval; $n = 6$; two-tailed Student's $t$ test, $P = 0.16$)

involvement of intracisternal glycine receptors in modulation of allodynia, we injected i.c. glycine and observed excitation and death in three mice; possibly attributable to co-activation of excitatory glutamate receptors.[11]

Acute injection of strychnine into the cisterna magna of mice has been previously shown to produce dynamic trigeminal mechanical allodynia restricted to the distribution of the trigeminal nerve,[8] and it has been shown to be supraspinal in origin.[8] These observations correlate well with the occurrence, type, and receptive field of dynamic allodynia observed in human trigeminal neuralgia. Our pairwise binary disequilibria design proved to be a useful technique for the identification of drug effects. Given the presence of glycine receptors in the spinal trigeminal nucleus,[13] the simplest explanation for the observed allodynia is that strychnine blocks central glycine receptors.[14-16] The spinal trigeminal nucleus is the most probable site of action of strychnine in our study, as strychnine applied to the trigeminal nucleus caudalis enhances responses in rostral nuclei evoked by innocuous facial tap.[17]

Direct action on glycine receptors to produce allodynia is unlikely for a number of reasons. Our direct addition of glycine to strychnine did not produce any antiallodynic effects at 20 μM or 200 μM, but 400 μM produced respiratory depression leading to death. Respiratory depression produced by glycine injection has been previously reported.[16] We have verified that the novel non-proteinogenic amino acid, isovaline, blocked strychnine-induced allodynia[18] through metabotropic receptors and not by acting on glycine receptors.[19] The prototypical $GABA_B$ receptor agonist, baclofen, has also been shown to be effective in animal models and in human trigeminal neuralgia,[20] and similarities between baclofen and carbamazepine have been reported.[21] Thus the antiallodynic action is considered to occur by restoring inhibition that is lost through strychnine-induced glycine receptor antagonism.

In addition to confirming the ineffectiveness of morphine against dynamic mechanical allodynia in the rat, this study in mice shows the effectiveness of carbamazepine's equipotent metabolite, CBZe, in alleviating intracisternal strychnine-induced allodynia.[5] These findings are consistent with clinical findings which show that carbamazepine is an effective first-line treatment[9] and that morphine is ineffective. The best justification for an animal model of human disease is a demonstration of the ability of the model to predict which drugs could be effective in humans. Since the novel experimental analgesic, isovaline, has been shown to be effective in this model, a test of this drug in humans will be one test of the predictive ability of this model. The present mouse preparation is simple, reproducible, economical, humane, and permits use of a vast repository of transgenic mice for advanced mechanistic studies and may be predictive of effective treatments in clinical trigeminal neuralgia.

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**Competing interests** None declared.

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