Safety and efficacy of BIIB074, a Nav1.7-selective sodium channel blocker, in trigeminal neuralgia: a double-blind, placebo-controlled, randomised withdrawal phase 2 trial

Zakrzewska JM, et al.

SUPPLEMENTARY APPENDIX: METHODS

Interim data review

An interim review of the response to BIIB074 in the first 10 patients completing the open-label period was planned in order to determine if the dose regimen of 150 mg tid was to be increased to 350 mg bid. The team reviewing the data consisted of an independent physician, three members of the contract research organization (biostatistician, medical monitor, project lead), two members of the sponsor (one the medical monitor), and the members of the diagnostic subcommittee of three external physicians who were also investigators in the study. The protocol allowed for the dose to be increased to 350 mg bid if 60% or fewer patients were randomised into the double-blind period of the trial. At the time of the interim review, seven of 10 (70%) open-label completers were eligible for randomisation (plus there was one dropout). Therefore the dose was not increased. No safety issues were raised during the interim review.

Protocol amendments

- All protocol amendments were made in accordance with the principles of Good Clinical Practice and were reviewed and approved by the relevant independent ethics committees or institutional review boards.
- Following review of demographic data for trigeminal neuralgia patients, the sponsor increased the maximum age for inclusion from 70 to 80 years. The rationale for this amendment was that trigeminal neuralgia is a condition that is associated with increasing age. Many patients are diagnosed in the 60-80 year age range and consequently the population of patients requiring treatment extends to patients aged 80 and older. By increasing the upper age limit, the population within the study would more closely reflect the final intended target population. (Amendment made May-August 2012).
- Data from the planned interim review indicated that some patients had a very marked response to BIIB074, such that they had no paroxysms in the final week of open-label treatment. The original protocol specified a treatment failure criterion of ≥50% increase in the number of paroxysms; consequently, a patient experiencing only one or two paroxysms during double-blind treatment could have met criteria for withdrawal. To avoid withdrawing such patients with only insignificant pain in the double-blind period, the withdrawal criteria were changed to require at least three paroxysms within a 7-day period in addition to a ≥50% increase in either the number or severity of paroxysms. The other withdrawal criteria remained unchanged. (Amendment made March-August 2013).
- The number of patients to be recruited to the open-label period of the study was increased from up to 40 to up to 70 patients, in order to achieve the target of randomising 30 patients with trigeminal neuralgia. (Amendment made August 2013).

Main inclusion and exclusion criteria

Inclusion criteria

- Male or female aged between 18 and 80 years (increased from 70 years in protocol amendment), with a diagnosis of trigeminal neuralgia.
- Female patients were to be of non-child bearing potential or agree to use an approved form of contraception.
- Male patients had to agree to use an approved form of contraception.
- Body weight ≥50 kg for men and ≥45 kg for women.
- Body mass index ≤34.9 kg/m^2.
- Patients had to be capable of giving written informed consent. Informed consent was obtained prior to the commencement of any study-related procedures.
- QTcF <450 ms in two of three electrocardiograms (ECG) conducted at screening.
- AST and ALT <2× upper limit of normal (ULN); alkaline phosphatase and bilirubin ≤1.5× ULN.
- Approved concomitant medications must have been stable for at least 3 weeks prior to Day 0.

Exclusion criteria

- Patients who were known non-responders to sodium channel blockers at therapeutic doses.
- Patients with causes for their facial pain other than specified in inclusion criteria.
- A positive pre-study drug screen.

- A positive history of HIV.
- A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening.
- History of any liver disease within the last 6 months, with the exception of known Gilbert's disease.
- History of excessive regular alcohol consumption within 6 months of the study.
- Patients with a history or risk of seizures or a history of epilepsy, head injury, or related neurological disorders.
- Patients with a history of uncontrolled or poorly controlled hypertension, with systolic blood pressure (BP) frequently exceeding 160 mmHg and/or diastolic BP frequently exceeding 100 mmHg, or patients who have BP ≥160 mmHg systolic and/or ≥100 mmHg diastolic at screening after repeated measurements.
- History or presence of significant cardiovascular, gastrointestinal, or renal disease, or other conditions known to interfere with the absorption, distribution, metabolism, or excretion of drugs.
- Patients with conditions known to affect cardiac conduction or a personal or familial history of Brugada syndrome.
- Pregnant or lactating females.
- History or presence of any clinically significant abnormality in vital signs / ECG / laboratory tests or any medical or psychiatric condition, which, in the opinion of the investigator, might have interfered with the study procedures or compromised patient safety.
- History of suicidal ideation and/or suicide attempts or clinical evidence of recent major depression (by medical history of the patient).
- Patients who were unable to maintain approved medications for their trigeminal neuralgia at a stable dose during the study, unable to refrain from excessive use of sedatives, or unable to comply with the prohibited concomitant medication restrictions (detailed below).
- History of hypersensitivity to BIIB074.
- Participation in a clinical trial and treatment with an investigational product within 5 half-lives or twice the duration of the biological effect of the investigational product (whichever was longer) prior to the start of this study.
- Exposure to more than four new chemical entities (medications for which no marketing authorisation has been obtained) within 12 months prior to the first dosing day.
- Where participation in the study would result in total donation of blood or blood products in excess of 500 ml within a 56-day period.
- Patient was mentally or legally incapacitated.
- Unwillingness or inability to follow the procedures outlined in the protocol.

Prohibited medications

- Prohibited medications included (but were not limited to):
  - Known or hypothesized sodium channel blockers and monoamine oxidase-B inhibitors (including lamotrigine, carbamazepine, oxcarbazepine, mexiletine, valproate, lidocaine, lacosamide, amitriptyline, topiramate, selegiline, rasagiline).
  - Medications that may adversely interact with a monoamine oxidase-B inhibitor, including monoamine oxidase inhibitors, antidepressants, opioids, and sympathomimetics.
- Permitted concomitant medications for the treatment of neuropathic pain had to have been stable for ≥3 weeks prior to the start of open-label treatment and maintained at stable dose. No new drugs for trigeminal neuralgia pain were permitted except for rescue medication of paracetamol (maximum dose 4g/day).

Statistical analysis for individual endpoints

All statistical tests were one-sided (assessing improvement with BIIB074 compared to placebo) and performed at the 5% level of significance (type 1 error). No adjustments were made for multiplicity.

Treatment failures (primary endpoint)

Treatment failure rates were compared between treatment groups using a one-sided Fisher's exact test with a type 1 error of 5%.

Time to treatment failure

Kaplan-Meier curves were plotted for each treatment group and groups were compared using the log-rank test; if possible, median time to failure with 95% CI was derived for each treatment.

Number and severity of paroxysms
For each week of the study, the patient's mean number of paroxysms per day and the mean severity of paroxysms were derived. Changes from baseline were summarised, where baseline was taken as the run-in week for the open-label phase and as Week 3 for the double-blind phase.

The mean number of paroxysms per day and the mean severity of paroxysms per week during the double-blind phase were analysed using general estimating equation (GEE) models. Linear regression over time (week) GEE models were fitted with a compound symmetry covariance matrix used for the Subject term in the model. Treatment and Week x Treatment interaction terms were included in the model such that a different intercept and linear slope were fitted for each treatment group. The data were assumed to be log-normally distributed, so a log link function was used. The treatment effect of BIIB074 was compared to that of placebo using a test for the difference between slopes.

The difference between treatment groups for the Week 7 change from baseline was estimated by the difference between the slopes multiplied by 4 (nominal 4 weeks of treatment). The associated 95% CI was also calculated. As a log link function was used, the treatment comparison (BIIB074 – placebo) is in the form of a ratio (BIIB074/placebo) when back-transformed.

Average daily pain score
The 24-hour average pain (11-point numeric rating scale [NRS]) was collected each day during the study. The patient's mean pain was calculated for each week and analysed using the same methods described above for the number and severity of paroxysms.

Clinician and Patient Global Impression of Change Scale (CGIC and PGIC)
Both categorical endpoints on Days 21 and 49 (or premature discontinuation) were summarised for each treatment group. Treatment groups were compared on Day 21 and Day 49 separately using the Wilcoxon Rank Sum Test using data over the whole scale.

Post-hoc analyses
Post-hoc analyses were conducted for the following:

- percentage change from baseline in number of pain paroxysms per day at the end of the double-blind phase
- change from baseline in severity of paroxysms at the end of the double-blind phase
- change from baseline in average daily pain score at the end of the double-blind phase.

Week 7 changes from baseline were analysed by analysis of covariance (ANCOVA) using different approaches for handling missing data - last observation carried forward (LOCF) and baseline observation carried forward (BOCF). For these analyses the run-in week scores were used for baseline, so the change from the start of the study was assessed. Baseline (run-in) and change from baseline during the open-label phase (Week 3–Week 0) were included as covariates in the ANCOVA model that included treatment as a factor.

The proportion of patients with ≥50% reduction in each of these measures from run-in to end of the double-blind phase was also calculated for the BIIB074 and placebo groups. All patients with missing data at Week 4 of the double-blind phase were considered non-responders.

Effects of BIIB074 on trigeminal ganglion neuron excitability
Pain in trigeminal neuralgia is thought to arise from abnormal ectopic firing of trigeminal ganglion neurons. A potential site and mechanism of action of BIIB074 in trigeminal neuralgia was explored through electrophysiological evaluation of the effect of graded concentrations (1 μmol/L and 10 μmol/L) of BIIB074 on threshold and evoked action potential firing of isolated rat small-diameter trigeminal ganglion neurons using current-clamp recordings.

Isolation and culture of trigeminal ganglion neurons
Trigeminal ganglia (TG) of Sprague-Dawley rat pups (postnatal day 0-5) were isolated and cultured as described previously.[1] Briefly, dissected ganglia were placed in ice-cold oxygenated complete saline solution (CSS), which contained the following (in mM): 137 NaCl, 5.3 KCl, 1 $MgCl_2$, 25 sorbitol, 3 $CaCl_2$ and 10 HEPES, pH 7.2. TG were then transferred to an oxygenated 37°C CSS solution containing 1.5 mg/ml collagenase A (Roche Applied Science) and 0.6 μM EDTA and incubated with gentle agitation at 37 °C for 20 min. This solution was exchanged with an oxygenated, 37 °C CSS solution containing 1.5 mg/ml collagenase D (Roche Applied Science), 0.6 μM EDTA, and 30 U/ml papain (Worthington Biochemicals) and was incubated with gentle agitation at 37 °C for 20 min. The solution was then aspirated and the ganglia were triturated in DRG media: DMEM/F12 (1:1) with 100 U/ml penicillin, 0.1 mg/ml streptomycin (Invitrogen), and 10% FBS (Hyclone), which contained 1.5 mg/ml bovine serum albumin (Sigma-Aldrich) and 1.5 mg/ml trypsin inhibitor (Sigma-Aldrich).

Current-clamp recording from TG neurons

Current-clamp recordings were obtained as described previously.[1-3] Whole-cell configuration was obtained in voltage-clamp mode before switching to current-clamp recording mode. The pipette solution contained (in mM): 140 KCl, 0.5 EGTA, 3 Mg-ATP, 5 HEPES, 30 dextrose, pH 7.3 with KOH (310 mOsmol/L). The extracellular bath solution contained (in mM): 140 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 15 dextrose, 10 HEPES, pH 7.3 with NaOH (315 mOsmol/L). BIIB074 was solubilised in DMSO to create 10 mM stock solution. On each experimental day, a working stock was prepared to give a final target concentration containing 0.1% DMSO. Solutions containing BIIB074 or DMSO control were applied using a perfusion system (Automate Scientific). Recordings were obtained from TG neurons based on the morphological criteria of small-diameter (20-28 μm) and round cell bodies. All recordings were obtained 2 days after plating. Resting membrane potential (RMP) and seal stability for each neuron were evaluated during a 30 s-long initial testing phase; neurons with RMP that varied by >10%, an initial RMP more depolarised than -40 mV, or with an RMP that exhibited an abrupt depolarisation indicating seal degradation at any point during the entire experiment were discarded. The cell input resistance and series resistance were also monitored during the recordings.

To evaluate the effect of BIIB074 on action potential firing of TG neurons, we measured the threshold for firing of the first action potential, a parameter that has been proposed to be influenced by Nav1.7.[3] Nav1.7 channels amplify sub-threshold membrane depolarizations; thus, inhibition of Nav1.7 should increase the minimum current necessary to fire an action potential. A before-and-after protocol was implemented in which excitability could be assessed prior to, during, and following exposure to BIIB074 so that each cell provided its own control. Current threshold for action potential generation was determined by a series of 200 ms depolarising currents in 5 pA increments. To quantify the effect on evoked repetitive firing, we measured the responses to graded depolarisations lasting 1000 ms. Evoked firing was examined using a series of 1 s-long current steps from 25 to 500 pA in 25 pA increments, with a 10 s interval between stimuli. For measurement of elicited spikes, transient membrane depolarisations with overshoot beyond 5 mV were counted as action potentials. After the evoked firing of the cell had been measured before exposure to BIIB074, the cell was exposed to 1 or 10 μM BIIB074 and evoked repetitive firing was re-evaluated. Only cells demonstrating multiple firing prior to application of BIIB074 (>5 action potentials at any given current step) were included for analysis of repetitive firing.

Application of BIIB074 1 μmol/L and 10 μmol/L to isolated rat trigeminal ganglion neurons was shown to significantly increase action potential threshold by 29.5% (figure S3, panels B,D) and 62.9% (figure S3, panels A,D), respectively. Further experiments measuring repetitive firing in response to a 1000 ms stimulus of increasing intensity demonstrated a significant reduction in firing following exposure to BIIB074 1 μM (figure S3, panels E-H) and 10 μM (data not shown). Of note, the 1-μM concentration of BIIB074 is similar to the maximum serum concentration of BIIB074 observed in the current Phase 2 study and a prior clinical trial (unpublished). Taken together, these results suggest that BIIB074, at concentrations close to the maximum plasma concentrations achieved in patients, has a direct inhibitory effect on the excitability of trigeminal ganglion neurons. These results suggest a probable mechanism of action consistent with current concepts of causation for trigeminal neuralgia.

References

1. Estacion M, Dib-Hajj SD, Benke PJ, et al. NaV1.7 gain-of-function mutations as a continuum: A1632E displays physiological changes associated with erythromelalgia and paroxysmal extreme pain disorder mutations and produces symptoms of both disorders. *J Neurosci* 2008; **28** (43): 11079-88. doi: 10.1523/JNEUROSCI.3443-08.2008.
2. Estacion M, Han C, Choi JS, et al. Intra- and interfamily phenotypic diversity in pain syndromes associated with a gain-of-function variant of NaV1.7. *Mol Pain* 2011; **7**: 92. doi: 10.1186/1744-8069-7-92.
3. Cummins TR, Rush AM, Estacion M, Dib-Hajj SD, Waxman SG. Voltage-clamp and current-clamp recordings from mammalian DRG neurons. *Nat Protoc* 2009; **4** (8): 1103-12. doi: 10.1038/nprot.2009.91.

SUPPLEMENTARY TABLES S1 and S2

Table S1. Open-label responder analysis

	Open-label phase, n (%) **(N=44, completed open-label)**
Treatment responders – mITT	29 (65.9)
≥30% reduction in number of paroxysms	25 (56.8)
≥30% reduction in severity	19 (43.2)
≥30% reduction in number of paroxysms & severity	18 (40.9)
PGIC much or very much improved	18 (40.9)

mITT = modified intent to treat; PGIC = Patient Global Impression of Change.
Patients can appear in more than one category.

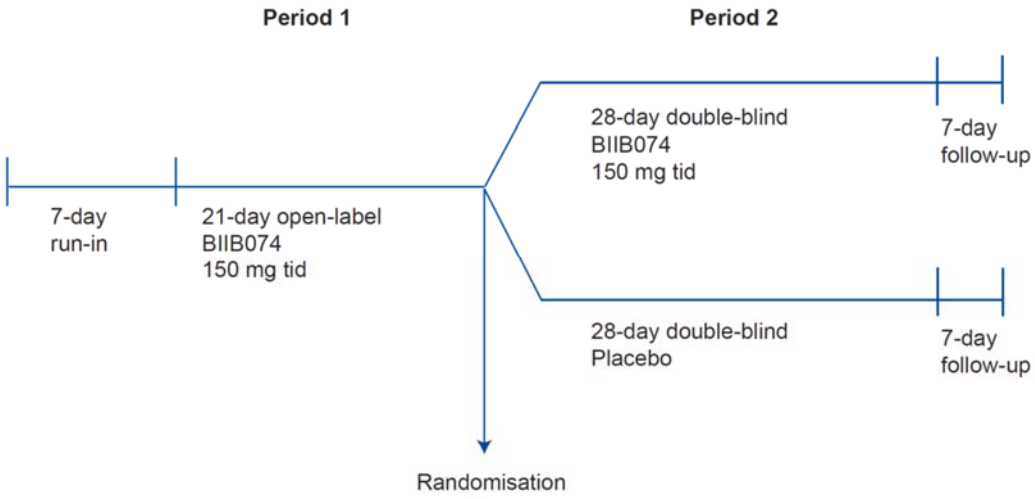
Table S2. Treatment failure analysis (mITT)

	Double-blind phase, n (%)	
	BIIB074 (N=15)	**Placebo (N=14)**
Treatment failure	5 (33.3)	9 (64.3)
Subcategories		
≥50% increase in frequency of paroxysms	1 (6.7)	5 (35.7)
≥50% increase in severity of paroxysms	0	6 (42.9)
PGIC of much or very much worse	0	3 (21.4)
Subjective lack of efficacy (double-blind phase)	4 (26.7)	7 (50.0)
Withdrawal of consent	1 (6.7)*	0

mITT = modified intent to treat; PGIC = Patient Global Impression of Change.
Patients may be included in multiple subcategories.
*One patient withdrew consent due to completion of the diary being too large a burden.

SUPPLEMENTARY FIGURES S1-S3

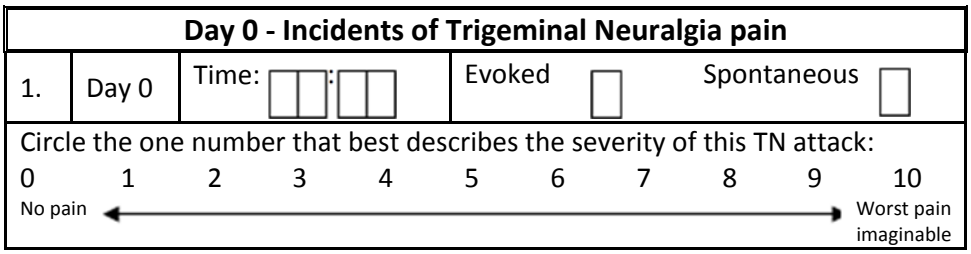
Figure S1. Study design



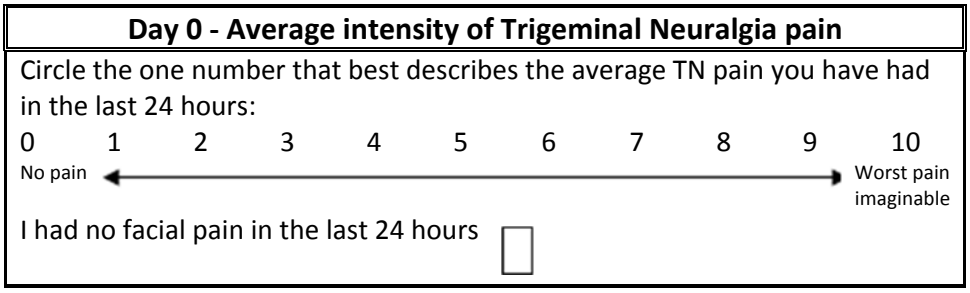
tid=3 times daily

Figure S2. PI-NRS for severity of individual paroxysms (A) and average daily pain (B)

A. Completed for each incident of trigeminal neuralgia pain

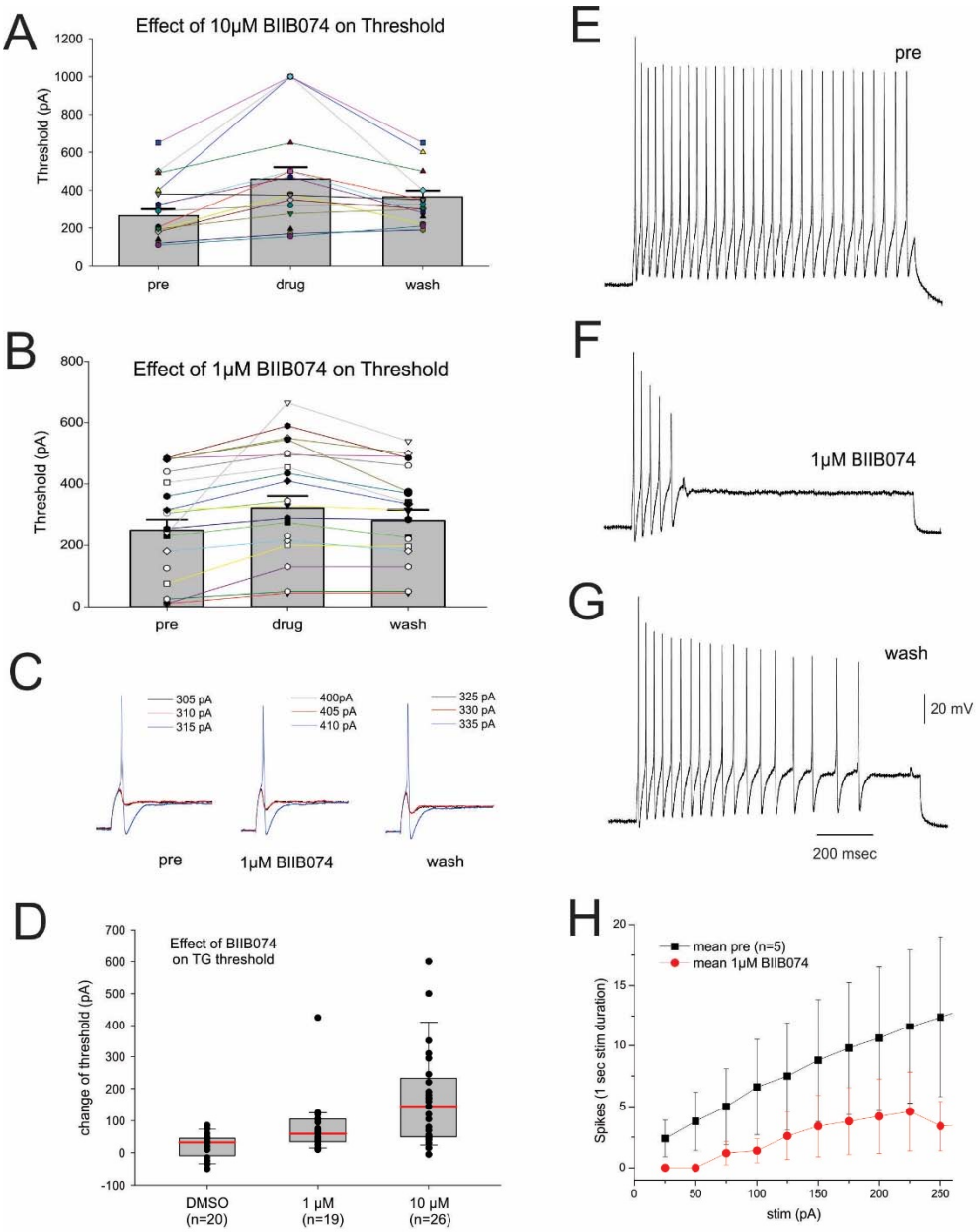


B. Completed at the end of each evening



PI-NRS=pain intensity numerical rating scale

Figure S3. Effect of BIIB074 on excitability in rat trigeminal ganglion neurons.



	Mean threshold pre	Mean threshold BIIB074/dmso	Mean threshold wash	Median delta threshold
0.1% DMSO (n=20)	316 ± 43 pA	340 ± 41 pA	341 ± 42 pA	32.5 pA
1μM BIIB074 (n=19)	274 ± 38 pA	355 ± 42 pA	308 ± 36 pA	60 pA*
10μM BIIB074 (n=26)	297 ± 30 pA	484 ± 55 pA	350 ± 29 pA	132.5 pA*

*p<0.05 compared to DMSO by ANOVA

(**A**) Effect of 10 μmol/L BIIB074 on action potential threshold (n=26). Control cells (pretreatment) displayed a mean threshold of 297 ± 30 pA SEM. BIIB074-treated cells showed a mean threshold of 484 ± 55pA SEM. The effect was reversible on wash-out of drug. Results were significant at p<0·0001 by paired t-test (Wilcoxon signed rank test). (**B**) Effect of 1 μmol/L BIIB074 on action potential threshold (n=19). Control cells (pretreatment) displayed a threshold of 274 ± 38 pA SEM. BIIB074-treated cells showed a threshold of 355 ± 42 pA. The effect was reversible on wash-out of drug. Results were significant at p=0·00033. Table shows numeric values for A and B together with control values for the 0·1% DMSO vehicle. (**C**) Representative recordings

from a single DRG neuron. In this cell 1 μmol/L BIIB074 raised the action potential threshold from 315 pA to 410 pA. The effect was reversible on washout. (**D**) Dose-response curve of the effects of 0.0 (DMSO), 1, and 10 μmol/L BIIB074 on action potential threshold. Red horizontal lines are medians. Boxes show 25th-75th percentile, error whiskers encompass 10–90th percentile. Note the clear dose-response relationship. Both 1 μM and 10 μM responses were significantly (p=0.009) increased compared to DMSO by analysis of variance (Kruskal-Wallis one-way analysis of variance on ranks). **BIIB074 reduces high-frequency firing of trigeminal neurons (panels E-G):** Response of a representative cell exposed to 1 μM BIIB074. (**E**) Repetitive firing in response to a 1000 ms 300 pA stimulus. (**F**) After 2 minutes of incubation with BIIB074 the number of action potentials elicited at the same current injection level was reduced. (**G**) After 2 minutes of washout, the number of action potentials partially recovered. **Averaged responses to 1 μM BIIB074 in cells displaying high firing (n=5) (panel H).** Comparison of the paired data (before and after application of BIIB074) demonstrated a significant difference (p=0.0019, analysis of variance of repeated measures) in response. Average responses did not show a significant effect of 0·1% DMSO. DMSO=dimethyl sulfoxide; DRG=dorsal root ganglion; SEM=standard error of the mean.