Published in final edited form as:

Cephalalgia. 2011 July ; 31(10): 1090–1100. doi:10.1177/0333102411410609.

Evaluation of cutaneous allodynia following induction of cortical spreading depression in freely moving rats

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Abstract

Background—Cortical spreading depression (CSD) is a wave of depolarization followed by depression of bioelectrical activity that slowly propagates through the cortex. CSD is believed to be the underlying mechanism of aura in migraine; however, whether CSD can elicit pain associated with migraine headache is unclear.

Methods—Awake, freely moving rats were monitored for both CSD events and behavioral responses resulting from dural-cortical pinprick and/or KCl injection to the occipital cortex.

Results—We observed tactile allodynia of the face and hindpaws, as well as enhanced Fos expression within the trigeminal nucleus caudalis (TNC) following CSD induced by KCl injection into the cortex, but not by pinprick. Application of KCl onto the dura elicited cutaneous allodynia and increased Fos staining in the TNC but did not elicit CSD events.

Conclusions—These data suggest that sustained activation of trigeminal afferents that may be required to establish cutaneous allodynia may not occur following CSD events in normal animals.

Keywords

CSD; migraine; nucleus caudalis; trigeminal; allodynia; fos

Introduction

Cortical spreading depression (CSD) is characterized by a wave of neuronal and glial depolarization followed by depression of electrical activity that slowly (3–6 mm/ min) propagates across the cortex and through most gray matter in the central nervous system including cerebellum, retina, hippocampus, caudate, thalamus and spinal cord. CSD has been shown to occur in many species including rabbits, rats, gerbils, cats, monkeys (1) and humans (2–5).

Functional imaging studies (6–9) have provided indirect but compelling evidence that CSD is the underlying mechanism of aura, a symptom experienced by approximately 10–20% of migraineurs (10–13). However, whether CSD events can precipitate pain that occurs during

a migraine or whether CSD and pain are parallel events is debated (14). As activation of the trigeminovascular system is thought to be a necessary event for generation of migraine pain (15), determining whether CSD events activate trigeminal afferents appears to be a logical step in defining the relationship between CSD and headache pain. Thus far, preclinical studies have shown that CSD events in anesthetized rats lead to alterations in blood flow in cranial vessels (16,17), enhanced plasma protein extravasation in the meninges, c-Fos activation in the trigeminal nucleus caudalis (TNC) (18), and long-lasting neuronal firing in meningeal nociceptors (19), suggesting that CSD produces neurochemical changes on the trigeminovascular system that could elicit headache. However, the effects of CSD in non-anesthetized rats are much less understood. Thus far the evidence suggests a disconnect between CSD and aversive or pain-like behavior (20,21). Behavioral changes in sensory thresholds reflecting activation of trigeminal afferents following CSD events in freely moving rats have not yet been demonstrated.

In addition to symptoms such as phonophobia, photophobia, nausea and vomiting, clinical studies reveal that about two-thirds of migraine patients experience cutaneous allodynia ipsilateral to their aura symptoms (22–24). Interestingly, cutaneous allodynia is highly prevalent (60–90%) in the peri-orbital area as well as extracephalic sites of migraine-aura patients (15,25,26). In rodent models thought to be relevant to migraine, sensitization of thalamic third-order neurons that receive convergent information from the head and forelimbs, as well as activation of brainstem structures with pain-modulatory functions such as the rostral ventro-medial medulla (RVM) and periaqueductal gray (PAG), have been implicated in central sensitization that may be responsible for migraine-associated cutaneous allodynia (22,27–29). While these data imply that sensitization of primary afferents and central trigeminovascular neurons in patients could result from the same mechanisms that initiate a CSD-induced aura, there is currently no satisfactory evidence for the association between aura and cutaneous allodynia (15).

The aim of these studies was to explore whether induction of a CSD event in a freely moving rat would lead to cutaneous allodynia, a likely consequence of trigeminal nucleus activation.

Materials and methods

Animals: Male Sprague Dawley rats (275–300 g) were purchased from Harlan (Indianapolis, IN) and were housed on a regular 12 h light/dark cycle (lights on at 07:00 hours) in a climate-controlled room with food and water ad libitum. All studies were performed while animals were on their light cycle between the times of 07:00 and 19:00 hours. All procedures were performed according to the policies and recommendations of the IASP, the NIH guidelines for laboratory animals, and by the IACUC recommendations of the University of Arizona.

Chemicals: KCl, saline and ketamine/xylazine were purchased from Sigma (St Louis, MO). Artificial CSF (aCSF) composition: NaCl, 145 mm; KCl, 2.7 mm; MgCl₂, 1mm; CaCl₂, 1.2mm; Na₂HPO₄, 2mm). pH was adjusted to 7.4 and the solution was filtered through a filter.

Implantation of recording electrodes: Rats were anesthetized with ketamine/xylazine (dose: 80:12 mg/kg i.p.) and fixed to a stereotaxic frame (Stoelting). Recordings were made with epidural Ag/AgCl electrodes prepared from 0.25 mm diameter Ag wire (A-M Systems, Inc., Everett, WA). Wire was flamed to produce spherical tips (1.0 mm diameter) and then coated with chloride. Electrodes were placed in burr holes through the skull made with manual drills, and two screws (#MPX-080-3F-1M, Small Parts Inc., Miami Lakes, FL) were placed

over the uninjured hemisphere (left). One screw served as a head-mount anchor. The other screw served as a ground electrode and it was located adjacent to the lateral ridge of the skull (7.5 mm posterior to bregma). The ground electrode was made by soldering a silver wire to the head of the screw. Lead electrodes were placed over frontal and parietal cortices (2.0 mm lateral, 1.5 mm anterior and 2.5 mm posterior to bregma, respectively) (Figure 1). A reference electrode was located posterior to lambda (11.5 mm from bregma). An additional burr hole (1.0 mm diameter) was made with a hand drill (DH-0 Pin Vise, Plastics One Inc., Roanoke, VA) to expose the dura at 6.5 mm posterior and 3.0 mm lateral (right) to bregma. A guide cannula (CSD cannula: 22 GA, #C313G, Plastics One Inc.) was fashioned to extend 0.5 mm from the pedestal and inserted into the hole with caution in order to avoid piercing the dura and sealed into place with glue. A dummy cannula (#C313DC, Plastics One Inc.) was used to avoid obstruction of the guide cannula. The free ends of the electrode wires were soldered to a multi-pin connector (Continental Connector, Hatfield, PA) and the assembly was fixed to the skull with dental cement. Rats were allowed to recover for 3 days before electrophysiological recordings and behavior studies were performed.

Injections

Cortical: Cortical injections were performed by employing a Hamilton injector (30 GA, #80308 701 SN) customized to project 1.0 mm into the brain. The injector is inserted through the CSD guide cannula to deliver 0.5 μ l of solution (1 M KCl or deionized water) or no solution (i.e. pinprick only) into the cerebral cortex. Artificial CSF (aCSF) was injected into the cortex (depth 1 mm).

Dura: Administration of solutions on the dura was performed using an injection cannula (28 GA, C313I) cut to the same size as the CSD guide cannula (0.5 mm). KCl (1 M; 10 μ l), aCSF or deionized water (vehicle for KCl) was slowly applied to the dura.

Electrophysiological recordings: Following recovery from surgery, rats were placed in suspended chambers (40 cm L $\times$ 49 cm W $\times$ 37 cm H) with wire mesh bottom (0.5 cm²) that allowed full access to the hindpaws during the recording period. These chambers were built to resemble Faraday cages, thus are appropriate for electrophysiological recordings. Via the multi-pin connector implanted on the head, rats were attached to an electro-cannular swivel (#CAY-675–6 commutator, Airflyte, Bayonne, NJ) stably fixed to the chamber's ceiling, which allowed the rat to move freely during the recording period. Baseline ECoG and DC recordings were obtained for 1 hour before any pharmacological treatment was performed. Only rats whose electrical recordings were stable were included in the experiment. Signals were recorded through shielded cables, input to separate channels for DC and AC amplification with a Grass (West Warwick, RI) Model 15 amplifier system (15A12 DC and 15A54 AC amplifiers), digitized at 100 Hz, and collected with EEG recording analysis software Gamma v.4.9 (Astro-Med, Inc. West Warwick, RI) (Figure 1). Because electrophysiological recordings were not available when these studies were initiated, we employed a separate group of animals (i.e. animals not connected to the CSD recording unit) to generate the behavior and Fos data (Figures 2 to 4). However, since then many behavior studies (Figure 5) have been performed during CSD recording to validate our original data.

Behavioral testing: Rats were acclimated for 1 hour before baseline measurements were made. Behavioral responses were determined by applying calibrated von Frey filaments perpendicularly to the midline of the forehead at the level of the eyes, or to the plantar aspect of the hindpaws, with sufficient force to cause the filament to slightly bend against the skin while held for approximately 5 seconds. A response was indicated by sharp withdrawal of the head or paw. Sensory thresholds were measured at 30, 60, 90, 120, 180, 240 and 300 minutes. The withdrawal thresholds were determined by Dixon's 'up and

down' method (30). Briefly, testing was initiated with a filament of certain force (1.0 g for the face and 2.0 g for the paw). In the absence of head withdrawal response, the immediate stronger filament was applied to the area; in the case of a withdrawal response, the next weaker filament was used. Maximum filament force was 8 g and 15 g for the face and hindpaw, respectively. As for measurement of paw withdrawal thresholds, testing began by application of the smallest (least amount of force) filament to one of the paws for 5 seconds. If no response was elicited, the same filament was then applied to the other paw for 5 seconds. When a withdrawal response was first observed by any of the hindpaws, the next filament (increasing in force) was used to determine the threshold by the 'up and down' method for that same paw only. We never assessed the frequency of responses by the right or left hindpaw, however we observed hindpaw hypersensitivity ipsilateral or contralateral to the right hemisphere (i.e. side of CSD induction), probably suggesting the development of central sensitization.

Immunolabeling. One week following surgery for cannula implantation, rats were split into three groups labeled 'surgery', 'water' and 'KCl'. Rats in the 'surgery' group underwent perfusion for tissue harvesting as described below. Rats in the 'water' and 'KCl' groups received cortical pinprick plus water (0.5 μ l) or KCl (1 M; 0.5 μ l), respectively. Hindpaw withdrawal thresholds were measured in all groups at 0.5, 1, 1.5 and 2 hours after injection. Facial sensory thresholds were not measured to ensure that Fos labeling observed would not result from facial stimulation. Animals with confirmed hindpaw allodynia were selected for perfusion and tissue harvesting for immunohistochemistry. Rats were deeply anesthetized with 100mg/kg of an 80:12 mixture of ketamine and xylazine and perfused transcardially with 250 ml of phosphate-buffered saline (PBS; 0.1 M; pH 7.4), containing 15,000 IU/L of heparin, followed by 4% paraformaldehyde in PBS for 20 minutes. The brainstem was removed, post-fixed in 4% paraformaldehyde overnight, and cryoprotected in 30% sucrose in PBS for 48 hours at 4°C. Transverse sections (40 μ m thick) were cut through the caudal medulla and collected serially in 0.1 M PBS for free-floating immunohistochemistry. The DAB immunostaining protocol was used for Fos labeling as described by Malick et al. (31). After three 10-minute washes in PBS, sections were pre-incubated for 30 minutes at room temperature in 0.3% hydrogen peroxide (Sigma). Following three 10-minute washes in PBS sections were incubated for 2 hours at room temperature in blocking buffer (0.1 M PBS, 0.25% Triton X-100 (Sigma), 3.0% normal goat serum). Sections were then incubated for 48 hours at 4°C in Fos primary antibody in PBS (Ab-5; specific to the Fos N-terminal domain, 1:20,000, Oncogene Science, San Diego, CA). After three 10-minute washes in PBS sections were incubated with secondary antibody in PBS for 2 hours at room temperature (biotinylated goat anti-rabbit IgG, 1:600, Vector Laboratories, Burlingame, CA). Following three 10-minute washes in PBS sections were reacted with avidin-biotin complex in PBS for 1 hour at room temperature (1:200, Vector Elite Kit, Vector Laboratories). After three 10-minute washes in PBS sections were reacted with 0.1 M PBS containing 0.04% 3,3'-diaminobenzidine tetrahydrochloride with urea and 0.01% hydrogen peroxide (DAB Sigma-Fast Kit, Sigma). The reaction was terminated after 9 minutes with three washes in PBS. Sections were then mounted onto gelatin-coated slides, dehydrated through ascending concentrations of ethanol and cover-slipped using Permount mounting media (Fisher Scientific).

Counting of Fos-labeled cells. Following a systematic sampling through the extension of the nucleus caudalis, most Fos immunoreactivity was identified in laminae I and II, 2–4mm below the obex. Thus cell counts were obtained from the same area for all testing groups. Four rats per treatment were used, and five sections from each animal were counted, totaling 20 sections per condition. A single side (chosen randomly) of each slice was counted. Data are expressed as mean number $\pm$ SEM of Fos-labeled cells per section. Significant

differences ($p < 0.05$) in cell counts were determined by ANOVA followed by Student–Newman–Keuls post hoc test.

Statistical analysis: All data were expressed as mean $\pm$ SEM. Comparisons among means or treatment groups were determined by one-way ANOVA. The post hoc Student–Newman–Keuls test was applied to find significant differences among means. A p -value < 0.05 was considered significant and is indicated by an asterisk (*).

Results

Cortical pinprick plus KCl injection elicited CSD in freely moving rats

Cortical pinprick plus KCl (1 M; 0.5 μ l) injection evoked CSD events in 24 of 28 (85.7%) rats. In almost every case, a single KCl injection evoked a single CSD event. These events occurred within 30–40 seconds following KCl injection, propagated at a speed of 6.63 ± 0.45 mm/min and manifested as DC-shifts with amplitude of 1.21 ± 0.11 mV and duration of 73.45 ± 2.73 seconds (Table 1); these characteristics are similar to those observed in prior reports of CSD events in awake animals (2–5). Cortical pinprick plus vehicle (deionized water; 0.5 μ l) produced CSD in 16 of 18 (88.9%) rats. Additionally, features of the CSD events following injection of deionized water were similar to those observed in the KCl-injected group. DC-shifts elicited in pinprick plus deionized water-treated rats showed amplitude of 1.28 ± 0.13 mV and propagation speed of 5.96 ± 0.66 mm/min. The duration of these events was 65.43 ± 2.61 seconds. Similar results were seen with aCSF in six animals (Table 1). Cortical pinprick alone (no KCl or water injected) produced CSD in 17 of 23 (73.9%) rats. The amplitude of DC-shifts evoked by cortical pinprick was 1.32 ± 0.18 mV and propagation speed was 6.07 ± 0.35 mm/min. Similar to the water group, the duration of CSD events evoked by pinprick was 62.86 ± 2.07 seconds. Neither the amplitude (one-way ANOVA, $p = 0.85$) nor the speed of propagation (one-way ANOVA, $p = 0.51$) of DC-shifts in the pinprick plus deionized water, pin-prick plus aCSF or pinprick alone groups was significantly different from the ones elicited by pinprick plus KCl. In addition, the incidence of CSD events did not differ significantly among treatment groups. Interestingly, the duration of CSD events in rats treated with pinprick plus KCl was significantly longer than in the pinprick plus deionized water, pinprick plus aCSF or pinprick alone group. Data are summarized in Table 1. Collectively, these data suggest that the pinprick is the initiating factor in eliciting the observed CSD events.

Cortical pinprick plus KCl, but not deionized water, nor aCSF injection produces cutaneous allodynia in freely moving rats

Following a 7-day recovery from surgery, rats were allowed to habituate for 1 hour before facial and hind-paw sensory baseline measurements were obtained. Rats received a cortical injection of KCl (1 M; 0.5 μ l), deionized water (0.5 μ l) or aCSF (0.5 μ l), and sensory thresholds were measured at 30, 60, 90, 120, 180, 240 and 300 minutes. Cortical pinprick plus KCl injection produced significant cutaneous allodynia in the face; withdrawal thresholds decreased from a baseline value of 8.0 g to 5.3 ± 0.5 g (one-way ANOVA; $p < 0.001$) (Figure 2A). Tactile allodynia was also observed in the hindpaw, evidenced by a significant decrease in sensory thresholds from 15 g to 8.3 ± 0.8 g (one-way ANOVA; $p < 0.001$) (Figure 2B). Allodynia in the periorbital region and hindpaw was observed as early as 30 minutes and peaked between 60 and 90 minutes after cortical KCl application. Interestingly, facial allodynia started to resolve between 120 and 180 minutes and facial thresholds returned to baseline values by 240 minutes. In the hindpaw, the decreased sensory threshold values were sustained throughout the 5-hour time course, returning to baseline values within 24 hours from the time of injection.

Separate groups of animals received pinprick and deionized water (Figure 3A, B) or aCSF (Figure 3C, D) injection in the cortex. Changes in sensory thresholds in the face or hindpaw evaluated over the 5-hour time course were not significantly different from baseline at any time point (one-way ANOVA; $p = 0.64$, $p = 0.12$, $p = 0.78$ and $p = 0.45$, respectively, Figure 3A, B, C, D).

Cortical pinprick plus KCl, but not deionized water, injection enhances Fos expression on the TNC

DAB staining indicated enhanced Fos immunoreactivity in the superficial laminae of the TNC of rats treated with cortical pinprick plus KCl (Figure 4). Rats subjected to cannula implantation but without any injections showed a significantly higher cell count for Fos 7 days following surgery (9.35 ± 1.00) than naïve rats (1.7 ± 0.4), reflecting the invasive nature of cannula implantation. In TNC from rats that received cortical application of deionized water (plus pinprick), Fos was present in 6.6 ± 0.6 cells, a value that was not statistically different from baseline in rats with cannula implantation and no injection. Two hours following pinprick plus KCl injection into the cortex, the number of Fos-expressing cells in the nucleus caudalis was significantly increased by approximately two-fold (19.0 ± 1.2 ; ANOVA followed by Student–Newman–Keuls post hoc test; $p < 0.0001$) (Figure 4).

KCl on the dura activates the trigeminal system in the absence of CSD events

Following 1 hour of baseline electrical recording, animals received a single application of KCl (1 M; 10 μ l) onto the dura and continued to be monitored for the occurrence of CSD events. A CSD event was observed in only one of eight rats that received dural KCl. As a positive control, at the end of the 5-hour recording period, CSD events were evoked in all eight animals by cortical injection of KCl (1 M; 0.5 μ l) (Figure 5A). Neither deionized water nor aCSF on the dura resulted in a CSD in either four or eight animals, respectively.

Dural application of KCl (1 M; 10 μ l), however, resulted in significantly increased Fos expression (19.15 ± 0.87) (Figure 5B) in the TNC, as well as behavioral signs of cutaneous allodynia (Figure 5C, D). Dural application of KCl (1 M; 10 μ l) produced significant mechanical allodynia in the face and hindpaw within 90 minutes and returned to baseline values by 300 minutes. Facial withdrawal thresholds decreased from a baseline value of 8.0 ± 0 g to 5.4 ± 1.1 g and hindpaw withdrawal thresholds decreased from a baseline value of 15.0 ± 0 g to 9.5 ± 2.2 g (one-way ANOVA; $*p < 0.05$) (Figure 5C, D) suggesting that dural KCl probably activates trigeminal fibers in the absence of CSD events. Neither deionized water nor aCSF on the dura (10 μ l) resulted in a significant change in facial or hindpaw thresholds from baseline ($n = 4$ or 8 animals, respectively).

Discussion

While accumulating evidence points to CSD as the underlying mechanism of aura in migraine patients, it is still not clear whether CSD elicits the headache associated with migraine. The studies presented here sought to investigate the relationship between the occurrence of CSD events and cutaneous allodynia, thought to reflect the development of central sensitization that may occur during migraine attack. Previous studies exploring the relationship between CSD events and activation of trigeminal afferents could not determine possible changes in sensory thresholds due to anesthesia. Here, we studied awake, freely moving rats in order to monitor for both CSD events and behavioral responses caused by pinprick with and without KCl or vehicle injection to the occipital cortex. Studies were performed on animals with minimal surgical trauma by not penetrating through the dura when inserting a cannula or CSD recording electrodes. Additionally, these events were

monitored 3 days following instrumentation of the animals in an effort to minimize any possible contribution of sensitization of afferent fibers produced by acute surgical injury.

Our data show that CSD events can be reliably evoked in awake rats following pinprick alone, pinprick plus vehicle or pinprick plus KCl injection into the cortex. However, cutaneous allodynia of the periorbital region of the face and of the hindpaws developed in a time-dependent manner following CSD evoked by pin-prick plus KCl injection, but not by pinprick alone or pinprick plus vehicle. Moreover, enhanced expression of Fos protein was found within the TNC 2 hours following cortical KCl injection, but not after pinprick alone or pinprick plus water. These results suggest that a CSD event alone is not sufficient to produce the sustained activation of trigeminal afferents that is probably required to establish central sensitization as reflected by cutaneous allodynia. These data must be interpreted cautiously. One possibility is that the generation of pain and aura during a migraine attack are parallel, independent events and not directly causal (15). However, it should be noted that the experiments here involved a CSD event in normal (i.e. 'non-migraineur') rats and the relationship between the CSD event and generation of pain may differ in migraine patients. Nevertheless, these data may also provide a possible explanation for the dissociation of headache pain and aura, the occurrence of headache pain in the absence of aura even in those patients who have a history of migraine with aura, and the development of aura hours to days after the headache pain has started.

By surgically implanting a guide cannula over the occipital cortex, with care to avoid piercing the dura mater, and allowing several days for recovery before any behavioral measurements, we were able to reliably elicit CSD in freely moving rats. In an attempt to reproduce previously reported strategies (32,33), CSD was evoked here by a single cortical injection of KCl. While previous studies performed multiple KCl injections or pinpricks over 1–2 hours (32–35), we focused on understanding the behavioral and neurochemical effects of a single evoking stimulus into the cortex.

CSD studies have traditionally been done in anesthetized animals, in which glass micropipette electrodes can be placed in cortical interstitial fluid to detect voltage changes in the 15–20 mV range (34,35). In freely moving awake rats, Hartings et al. (36) demonstrated that Ag/AgCl epidural macroelectrodes may also be employed to successfully detect CSD, yet in these conditions the events are in the 1–5 mV range. In agreement with previous work (34,36), our epidural electrodes reliably detected CSD waves with mean amplitude of 1.2 mV, and characteristic propagation speed and duration of ≈ 6.0 mm/min and 70 seconds, respectively, validating our recording system as a means to monitor the occurrence of CSD events in awake rats.

We observed that cortical pinprick alone evoked CSD in 17/23 (73.9%) rats. Following treatment with cortical pinprick plus KCl injection, pinprick plus water or aCSF injection, CSD events were observed in 24/28 (86%), 16/18 (89%) and 5/6 (83%) rats, respectively. The incidence of CSD events as well as their amplitude and propagation speed was not significantly different among treatments. As reported previously (37), a significant difference was found in duration of events, in which pinprick plus KCl evoked CSD events of longer duration than those elicited by cortical pinprick alone or pinprick plus water. Although similarly effective in eliciting CSD events, chemical stimuli such as KCl have an extended duration of action when compared to electrical and mechanical stimulation (38). It is possible that as KCl slowly disperses, it depolarizes a larger population of cells, hence more neurons are recruited to undergo CSD, which could account for the lengthened time for recovery from depression.

Expression of the proto-oncogene c-Fos has been widely used as a surrogate marker on neuronal activation including animal models of migraine (39). That CSD propagates through ipsilateral cortex, activating Fos expression in ipsilateral TNC has been extensively demonstrated (17,21,32,33). Studies here sought to use Fos expression in the TNC as a marker that in a CSD model employing awake animals, some well-known consequences of CSD can be characterized. In keeping with previous reports (32,40), results indicate that brainstem harvested from rats that showed significant tactile hypersensitivity 2 hours after cortical KCl injection revealed an enhanced number of Fos-expressing cells within the TNC, suggesting activation of these second-order neurons following CSD. However, Fos immunoreactivity in the TNC of animals treated with cortical injection of water or pinprick alone was negligible, despite the occurrence of similar CSD events under those conditions. Ingvarlsen and colleagues (33) found that the amount of Fos-expressing cells in the TNC was positively correlated to the number of KCl injections and not to the number of CSD events, proposing that the trigeminal system was activated by KCl and not by CSD. Taken together, our results may suggest that KCl, rather than a CSD event, directly activates the trigeminal system, though it is also possible that the lengthened CSD event produced by cortical KCl, but not water or pinprick alone, is sufficient stimulus for long-lasting activation of meningeal afferents.

Earlier studies indicated that spreading depression in the cortex (17,19) or hippocampus (40) may result in increased meningeal or cortical blood flow and plasma extravasation in anesthetized rats. Here, we extended those findings by showing that, following a CSD event elicited by cortical KCl injection plus pinprick, freely moving rats developed cutaneous allodynia in the face. We also note that the allodynia observed following KCl was generalized and could be observed following probing of the hindpaws. Thus, cortical KCl injection may promote the development of central sensitization, a phenomenon shown to underlie cutaneous allodynia associated with headache in models of dural inflammation (27–29,41). In agreement with our results on Fos expression, rats treated with cortical injection of water or pinprick alone did not develop cutaneous allodynia, although CSD events were detected in the majority of animals, again suggesting that KCl, rather than CSD, activates meningeal afferents and leads to pain behavior in rats. As these studies targeted the measurement of generalized hypersensitivity within the midline of the periorbital region and hindpaw, it is possible that allodynia occurring in other regions following cortical pinprick may have been missed. The lack of direct correlation between CSD events and aversive (20) and pain behavior (21) in awake rats has been previously demonstrated. Together, these data also translate to reports in humans that experience migraine without CSD (i.e. aura) and migraineurs that experience CSD without a headache, or that headache in humans may be less severe and short-lasting if preceded by aura (42,43).

With the goal of testing if KCl can activate the trigeminal system independently from CSD events, we showed that generalized cutaneous allodynia and Fos expression in the TNC were observed following application of KCl to the dura, in spite of the fact that no CSD events were recorded. These observations suggest that cortical KCl may diffuse sufficiently to activate trigeminal afferents directly and result in cutaneous allodynia yet not provoke a measureable CSD event similar to when KCl was directly injected into the cortex. Although c-Fos immunohistochemistry over the entire cortex was not assessed to completely rule out cortical activity, the lack of measureable electrical activity suggested no CSD event.

The findings that CSD produced by a cortical pinprick alone did not result in cutaneous allodynia in the face or hindpaw, or enhanced Fos expression in the TNC, suggest that a CSD event alone is not sufficient to lead to sustained activation of the trigeminal system that is likely to be required to establish cutaneous allodynia, at least in normal animals. Whether

CSD events may activate trigeminal afferents in migraineurs is unknown and awaits further investigation.

Acknowledgments

Funding

This research was supported by GlaxoSmithKline and the National Institutes of Health NS069572.

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Figure 1.

Schematic of skull indicating positions of electrode and cannula placement; ECoG and DC traces from freely moving rats after receiving a cortical injection of KCl via the cannula clearly demonstrating a wave of CSD from electrode 2 (DC 2) of the parietal region and moving to electrode 1 (DC 1) of the frontal cortex.

Figure 2.

Behavioral mechanical testing in animals after the application of KCl (1 M; 0.5 µl) into the occipital cortex. KCl induced periorbital facial allodynia (A) and hindpaw allodynia (B) within 30 minutes after pinprick through the dura and KCl injection. Facial withdrawal thresholds returned to baseline within 300 minutes after KCl injection, however paw withdrawal thresholds were still below baseline thresholds at 300 minutes. *Significant ($p < 0.05$) differences from baseline.

Figure 3.

Behavioral mechanical testing in animals after the application of deionized water (0.5 µl) or artificial cerebral spinal fluid (aCSF) into the occipital cortex. A pinprick with water injection into the occipital cortex did not result in significant facial (A) or hindpaw (B) allodynia over a 300 minute period. A pinprick with aCSF injection into the occipital cortex did not result in significant facial (C) or hindpaw (D) allodynia over a 300 minute period. Significance was tested for differences from baseline ($p < 0.05$).

Figure 4.

Medullary sections (40 μm thick) were harvested from naive rats, rats with dural cannulation only (surgery), and rats receiving either a pinprick plus water (vehicle for KCl) or KCl (1 M, 0.5 μl) in the occipital cortex. Sections were prepared for diaminobenzidine tetrahydrochloride staining to visualize Fos expression, and the numbers of Fos-positive profiles within the trigeminal nucleus caudalis (TNC) were counted. Each group represents a total of 20 sections obtained from four rats. The results indicate that cannulation surgery alone produced an increase in trigeminal Fos expression compared with the naive rats, and pinprick with water was similar to surgery alone levels. Cortical administration of KCl

produced a significantly greater increase in Fos expression over pinprick with water.

*Significant ($p<0.05$) differences among mean counts.

Figure 5.

(A) The mean number of CSD events over a 1 hour period in animals administered either deionized water, aCSF or KCl (1 M, 10 μ l) onto the dura as well as KCl into the cortex as a positive control. Unlike KCl injection into the cortex, KCl administered onto the dura did not result in a significant number of CSD events. Water or aCSF on the dura had no effect. (B) Fos expression in medullary sections from naïve rats, rats with dural cannulation receiving water only, and rats receiving KCl (1 M, 10 μ l) on the dura. The results indicate that cannulation surgery and water alone produced an increase in trigeminal Fos expression compared with the naïve rats, and KCl administration significantly enhanced Fos expression. **Significant ($p < 0.001$) differences among mean counts. Each group represents a total of 20 sections obtained from four rats. (C) Behavioral mechanical testing in animals after the application of KCl (1 M, 10 μ l) onto the dura. The application of KCl to the dura resulted in significant facial and hindpaw allodynia (D) within 90 minutes after application and lasted up to 240 minutes. *Significant ($p < 0.05$) differences from baseline.

Table 1

Frequency of CSD and parameters of DC-shifts in each treatment group

	Treatment groups			
	Pinprick	Pinprick + water	Pinprick+aCSF	Pinprick+ KCl
Frequency	17/23 (73.9%)	16/18 (88.9%)	5/6 (83.3%)	24/28 (85.7%)
Speed of propagation (mm/min)	6.1 $\pm$ 0.35	6.0 $\pm$ 0.7	6.1 $\pm$ 0.9	6.6 $\pm$ 0.4
Amplitude (mv)	1.3 $\pm$ 0.2	1.3 $\pm$ 0.1	1.3 $\pm$ 0.2	1.2 $\pm$ 0.1
Duration (s)	62.9 $\pm$ 2.1	65.4 $\pm$ 2.6	63.7 $\pm$ 2.9	73.4 $\pm$ 2.7 *

*
 $p < 0.05$.