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The effects of repeated nitroglycerin administrations in rats; modeling migraine-related endpoints and chronification

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HIGHLIGHTS

- Repeated NTG administration induces clinically-relevant migraine endpoints in rats.
- NTG vehicle of PG/EtOH alters migraine endpoints.
- Spontaneous tactile allodynia was quantified in a novel arena apparatus.
- This paradigm simulates features of episodic migraine and its chronification.

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ABSTRACT

Background: Rodent models typically use a single nitroglycerin injection to induce migraine, yet migraine in clinical populations presents as recurrent episodes. Further, these models quantify behavioral endpoints that do not align with the clinical features of episodic migraine or migraine chronification and therefore may limit translational relevance.
New method: Rats received 5 nitroglycerin (10 mg/kg/2 ml), propylene glycol/ethanol vehicle, or saline injections every third day over 15 days. Behavioral endpoints were assessed 110 min post nitroglycerin administration and included time spent light/dark chambers for photophobia as well as activity, facial pain expressions, and tactile allodynia.
Results: Animals administered nitroglycerin displayed photophobia, decreased activity, and increased facial pain expression. Similar alterations in photophobia and activity were seen in the vehicle treated animals, but these tended to diminish by the 4th or 5th injection. The presentation of spontaneous tactile allodynia was observed in the nitroglycerin group by the 5th episode.
Comparison with existing methods: Most NTG migraine models entail a single NTG administration and quantification of evoked allodynia. This paradigm employs recurring NTG episodes and clinically-relevant measures of photophobia, hypoactivity and facial grimace endpoints as well as introduces a novel arena apparatus to quantify spontaneous allodynia.
Conclusions: This repeated NTG procedure and endpoint measures aligns with the frequency and clinical presentation of episodic migraine and its chronification, respectively. Further, propylene glycol ethanol vehicle contributes to migraine endpoints.

1. Introduction

Migraine is the third most common medical condition in the world (Vos et al., 2012) and produces significant disability and reduced quality of life (Leonardi et al., 2005; Vos et al., 2012). Migraine presents as unilateral, throbbing head pain and increased sensitivity to light and sound that is worsened by activity. Migraine also presents as recurrent episodes interspersed by pain-free

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intervals (Headache Classification Committee of the International Headache Society (HCC), 2013). As migraine episodes recur, allodynia may spontaneously present during activity (Lipton et al., 2008). Such spontaneous allodynia is not a diagnostic symptom of migraine but rather a marker of migraine chronification (Burstein et al., 2004; Louter et al., 2013). Despite its high prevalence, migraine remains undertreated and its pathophysiology poorly understood (Lipton et al., 2007). These challenges stem in large part from a paucity of valid animal migraine simulations (Olesen and Jansen-Olesen, 2012).

One rodent model of migraine (Buzzi and Tassorelli, 2010) is based on the observation that nitroglycerin (NTG[1]) taken for angina triggers headache (Bates et al., 2010) and induces migraine in migraineurs (Thomsen et al., 1994). However, none of these NTG models fully align with clinical presentation of migraine as indexed by either induction frequency or behavioral endpoints (Mathew et al., 2004; HCC, 2013). Most, but not all (Pradhan et al., 2014), use a single NTG administration to induce migraine (Tassorelli et al., 1997) and all use evoked tactile allodynia via von Frey tests as a behavioral endpoint (Andreou et al., 2010; Edelmayer et al., 2012; Costa et al., 2005; Pradhan et al., 2014). These methodological characteristics may account for the poor translational relevance with NTG migraine models.

Recent research by Sufka et al. (2016) addressed these migraine model concerns by determining whether multiple NTG administrations affect behavioral endpoints that align with the International Classification of Headache Disorders (ICHD) diagnostic criteria (HCC, 2013). In this model, rats were given 1, 3 or 5 injections of NTG. Endpoints quantified after the final NTG episode for each group included measures of photophobia and activity in a light/dark box, and facial pain expressions (Rat Grimace Scale, Sotocinal et al., 2011). Multiple, but not single, NTG administrations produced photophobia, decreased activity, and increased pain intensity in facial expressions. Further, sumatriptan, an abortive migraine treatment, dose-dependently reversed two of these three clinically-relevant endpoints (motor activity, facial expressions of pain; Sufka et al., 2016).

Although this recurring NTG migraine model aligns with clinical presentation of human migraineurs, a number of procedural components require further investigation. First, while multiple NTG episodes alter a number of clinically-relevant endpoints (Sufka et al., 2016), how these signs progress after each NTG episode is unknown. Second, rodent migraine models report endpoints of evoked allodynia (e.g., via Hargreaves or von Frey procedures; Bates et al., 2010); we believe it may be possible to quantify non-evoked (i.e., spontaneous) allodynia during spontaneous activity (Mogil and Crager, 2004) under a migraine episode using a modified open field type apparatus to track ongoing behavior as has been done in a model of chronic constrictive injury (Murai et al., 2016). Finally, existing migraine models compare NTG concentrations of either 10 mg/kg/2 ml or 10 mg/kg/10 ml to saline as a control group (Tassorelli et al., 1997; Bates et al., 2010; Pradhan et al., 2014). Whether NTG vehicle contributes to clinically-relevant endpoints in a recurring migraine model (Sufka et al., 2016) requires using an NTG vehicle as an additional control group. Thus, this study sought to address each of these considerations by a) tracking endpoints of photophobia, motor activity and facial pain expressions subsequent to each NTG episode, b) introducing a novel apparatus for quantifying non-evoked allodynia that may occur spontaneously during normal exploration, and c) adding the vehicle for NTG as a control.

[1] Abbreviations: NTG-Nitroglycerin; ICHD-International Classification of Headache Disorders; PG-propylene glycol; Et/OH- ethanol; IP- intraperitoneal; L/D-light dark

2. Methods

2.1. Subjects

A total of 30 male Sprague Dawley rats (175–199 g; Envigo, Indianapolis, IN), were pair-housed and received the same treatment conditions. Animals were maintained under a 12:12 h light-dark cycle in a temperature and humidity controlled vivarium, were used in this study. Food and water were available ad libitum. Animals were handled twice daily (7d) prior to experimental manipulations to reduce experimenter-related stress. All experimental procedures were approved by the Institutional Animal Care and Use Committee (Protocol #13-023). Both male and female researchers were present for each test session.

2.2. Drug administration

The NTG stock solution contained 5 mg/ml NTG dissolved in 30% alcohol, 30% propylene glycol, and water (American Regent; Shirley NY). NTG was administered in a volume of 2 ml/kg to achieve a dose of 10 mg/kg. This concentration was chosen over the more dilute (10 mg/10 ml) but larger volume (10 ml/kg) administration due to animal welfare concerns, as animals in this study would be receiving 5 NTG injections over a 2 week period. Two control groups were used: a vehicle containing 30% propylene glycol (PG; Sigma Aldrich, St. Louis, MO), 30% 200 proof ethanol (Et/OH; Decon laboratories, King of Prussia, PA) in saline; and a sham control group receiving saline (0.9%). All test articles were administered intraperitoneally (IP) in a volume of 2 ml/kg. Induction of episodic migraine episodes consisted of 5 NTG injections administered every third day over 15 days. Rats acclimated in the testing room for at least 1 h in their home cages prior to behavioral testing. Behavioral testing started 30 (RGS), 110 (light dark box), and 130 (tactile arena) min post NTG injections. This time point was chosen to be consistent with prior recurrent NTG migraine studies that showed NTG effects on these behavioral endpoints (Sufka et al., 2016; Pradhan et al., 2014)

2.3. Rat grimace scale

The Rat Grimace Scale was utilized to quantify pain by examining facial features of a rat during a pain state. Images of the rat's facial features were taken 30, 60, and 90 min post NTG administration. Multiple photos were taken manually with a 5 megapixel camera. Pictures that best depicted the all 4 facial features were scored. These time points were chosen as migraine is a continuous process with two distinct peaks in intensity with the first peak appearing 15–30 min post NO administration (Olsen, 2008). Further, studies with Sufka et al. (2016) demonstrated no measurable effects were found at 60–90–120 post NTG administration. The most robust and consistent RGS were found at 30 min and thus only these data are presented. Rats remained in their home cages to reduce experimental stress. Following the experiment, unlabeled images were displayed on a computer monitor for scoring by two trained research assistants who were blinded to condition. Images were scored for four features and included orbital tightening, nose/cheek flattening, and ear and whisker changes using a scale of 0–2 (not present, moderate, obvious). Scores were averaged across the two raters to produce an overall pain score for each rat. Inter-rater reliability was $\alpha = 0.85$.

2.4. Light/dark box

Light Dark (L/D) box testing for photophobia and activity was conducted in a two chamber condition place apparatus (Med-Associates, St. Albans, VT) with chamber dimensions of 25.5 cm × 21.0 cm × 20.9 cm. Black construction paper was taped