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Behavioral study of non-evoked orofacial pain following different types of infraorbital nerve injury in rats

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HIGHLIGHTS

- Face grooming after different types of infraorbital nerve ligation was compared.
- Tight ligation of the infraorbital nerve has long lasting effects on face grooming.
- Formalin grooming does not predict grooming after infraorbital nerve ligation.
- Repeated testing increases statistical power and reduces the number of animals.

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ABSTRACT

Directed isolated face grooming following unilateral chronic constriction injury to the infraorbital nerve (IoN-CCI) is a unique measure of spontaneous neuropathic pain. Variability between rats and the limited duration of the increased face grooming behavior has hampered its usefulness. We studied three possible sources of variability: variations in surgery, pre-existing differences in nocifensive behavior between the rats and variation in time. Three different types of IoN lesion were performed: loose ligation (CCI), tight ligation (CCI-T) and partial tight ligation (PTL, Seltzer method); the latter two offer greater surgical standardization. Face grooming behavior following IoN injury, on the one hand, and during the orofacial formalin test, on the other hand, was analyzed and correlated. Significant differences in isolated face grooming behavior were found between the IoN groups. Interestingly, CCI-T rats continued to show significantly increased isolated face grooming for the duration of the experiment, i.e., up to 32 days post-operative, whereas CCI animals were no longer significantly different from sham animals after two weeks. Surprisingly, PTL operated rats only showed minor effects. Variability was not smaller in the CCI-T or PTL group. Face grooming behavior after IoN lesion was poorly correlated to that in the orofacial formalin test. It is therefore unclear if pre-existing behavioral differences between animals are a major cause of variability in the IoN-CCI model. Finally, repeated testing showed significant variability in time. It is concluded that tight ligation of the IoN nerve has long-lasting effects on face grooming behavior and that part of the variability in face grooming behavior may be reduced by performing repeated testing.

1. Introduction

Neuropathic pain substantially reduces patients' quality of life [1,2]. It is a chronic condition that is challenging to treat [3]. Symptoms such as allodynia, hyperalgesia, paresthesia and dysesthesia vary considerably among patients and are largely resistant to treatments with commonly prescribed analgesics [4]. Animal models are used to examine the efficacy of existing and newly developed drugs and to identify the pathophysiological mechanisms involved in the development and maintenance of neuropathic pain. Most of these studies (90%; [5]) have focused exclusively on measuring hypersensitivity: mechanically (pinch/pressure) and thermally (heat/cold) evoked nocifensive behavior (e.g. vocalization, withdrawal responses). Only a very limited number of studies have attempted to measure spontaneous chronic pain. This is in contrast with the fact that the primary complaint of pain patients is ongoing pain, not hyperalgesia or allodynia [6]. Furthermore, based on existing evidence, the pathophysiology of hypersensitivity may be different from that of spontaneous chronic pain [5,7]. As a result, drug screening based on evoked pain will only have a limited predictive validity for efficacy in clinical neuropathic pain syndromes. To date, directed, isolated face grooming following infraorbital nerve (IoN) ligation is the best indicator available of spontaneous neuropathic pain in rats. Unfortunately, variability between rats and the limited duration

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of the increased face grooming behavior has limited its usefulness in the study of neuropathic pain and the development of new drugs [8].

Lack of surgical standardization may be a major source of such variation. The criterion that is used for determining the amount of nerve constriction was first described by Bennet and Xie [9] and adopted by Vos et al. [10]. It states that the ligatures must reduce the diameter of the nerve by a just noticeable amount and should retard, but not interrupt the circulation through the superficial vasculature. Although this is a valuable guideline, it may still be the cause of variation, especially in the IoN model, considering the poor accessibility to the nerve. Several models of nerve ligation have attempted to overcome this problem, e.g. by tightly ligating (and cutting) one or more of several branches of a nerve [11], by tightly ligating one or more spinal nerves [12], or by tightly ligating part of the nerve [13]. Tightly ligating a nerve to the point where you cannot further apply force is expected to have greater standardization. In the present study, the effects of loosely ligating the IoN were compared to those of tightly ligating the entire or part of the IoN.

Secondly, it is possible that differences in nocifensive behavior pre-exist between rats and are thus inducing variability [14,15]. In the present study, it is hypothesized that these differences may become apparent in the orofacial formalin test as well as in the IoN ligation model, i.e., if an animal prefers face grooming as nocifensive behavior in the IoN model, this may also be the case in the formalin test. Formalin injection in the upper lip has been shown to induce biphasic directed face grooming behavior in rats [16,17]; these data can thus be correlated to face grooming data in the IoN model. As a result, it may be possible to predict (and possibly select) which animals will be (un)responsive to IoN ligation based on data obtained in the orofacial formalin test.

Finally, it is well known that trigeminal neuropathic pain is often characterized by paroxysmal pain episodes [18,19]. It is possible that also in the IoN model, spontaneous pain may be subject to variation in time. Therefore, in the present protocol, animals were tested twice daily instead of only once.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Charles River, N = 48, weighing 275–300 g at arrival) were housed in solid-bottom polycarbonate cages in a colony room with a humidity of 45 ± 5% and a room temperature of 21 ± 1 °C. Water and food were available ad libitum. Rats were kept under a reversed 12:12 h dark/light cycle (lights on at 20 h). Animals were treated and cared for according to the guidelines of the Committee for Research and Ethical Issues of IASP [20]. The protocol was approved by the institutional Ethical Committee.

Rats were allowed to acclimate for 8 days to the housing conditions before the formalin test. Rats were habituated to the formalin test procedure one and two days before the formalin test. A period of 7 days was respected between the formalin test and pre-operative observations. Rats were habituated to the test procedure every day for three days before pre-operative testing. Habituation and testing were conducted in a darkened room (light provided by a 60 W red light bulb suspended 1 m above the observation area) with a 45 dB background noise. Rats were individually transported from the colony to the test room (15 s trip) in a covered plastic cage without bedding (l × w × h: 24 × 14 × 17 cm).

2.2. Surgery

The unilateral ligation of the infraorbital nerve was performed as described elsewhere [10]. Rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and treated with atropine (0.1 mg/kg, i.p.). Surgery was performed under direct visual control using a Kaps operating microscope (×10–25). The rat's head was fixed in a stereotaxic frame and a mid-line scalp incision was made, exposing the skull and nasal bone. The infraorbital part of the left IoN was exposed using a surgical procedure similar to that described earlier [21,22]. The edge of the orbit, formed by the maxillary, frontal, lacrimal and zygomatic bones, was dissected free. To give access to the IoN, the orbital contents were gently deflected with a cotton-tipped wooden rod. The IoN was dissected free and two chromic catgut ligatures (5-0) were loosely tied around the IoN (2 mm apart). The ligatures reduced the diameter of the nerve by a just noticeable amount and retarded, but did not interrupt the circulation through the superficial vasculature. The scalp incision was closed using polyester sutures (4-0; Ethicon, Johnson & Johnson, Belgium).

Tight ligation of the infraorbital nerve (CCI-T) was performed using the same procedure as used for the loose ligation, except that only a single catgut ligature (5-0) was tied around the IoN as tightly as possible.

The same procedure was followed for the partial tight ligation (PTL), except that approximately one third to one-half the diameter of the nerve was tightly ligated with 6-0 Mersilk suture by passing the needle under the nerve and then up through the middle.

In sham operated rats, the IoN was exposed using the same procedure, but the exposed IoN was not ligated.

2.3. Study design

Rats were randomly assigned to one of four experimental groups: 12 rats received a loose IoN ligation (CCI), 12 rats received a tight IoN ligation (CCI-T), 12 rats received a partial tight ligation (PTL), and 12 rats received a sham operation.

Eight days before IoN surgery, half the rats in each group (i.e., N = 6) received an injection with formalin in the snout, the other half received a saline injection in the snout.

2.4. Behavioral testing

2.4.1. Formalin test

Rats were s.c. injected with 50 μl of formalin (1.5%) or saline near the center of the right – i.e. contralateral to the operated IoN – vibrissal pad. Face grooming behavior was recorded during a 35 min period.

2.4.2. IoN ligation

Face grooming behavior was observed on pre-operative day −1 and on post-operative days +3, +5, +7, +10, +19, +25 and +32. Behavior was videotaped twice for 10 min (once in the morning between 9 h and 11 h; and once in the afternoon between 14 h and 16 h). Videotaped behavior was analyzed by an experimenter who was blind to the experimental group of the rat.

The amount of time spent on face grooming (i.e., movement patterns in which paws contact facial areas; see [8]) was determined using a stopwatch. A distinction was made between isolated face grooming and face grooming during body grooming [23]. If a sequence was neither preceded nor followed by body grooming (i.e., movement patterns in which the paws, tongue, or incisors are brought in contact with a body area other than the face or the forepaws, see [10]), the episode was categorized as *isolated face grooming*. The amount of time spent on isolated face grooming was determined as the sum of time spent on isolated face grooming episodes recorded during the observation period. If body grooming was present before or after a sequence of face grooming actions, the episode was categorized as *face grooming during body grooming*. The amount of time spent on face grooming during body grooming was calculated in the same way as for isolated face grooming.

2.5. Statistical analysis

Data were analyzed using IBM SPSS Statistics 20 software. Data are expressed as mean ± S.E.M. and were analyzed by means of a repeated