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ATTENUATION OF NEUROPATHIC MANIFESTATIONS BY LOCAL BLOCK OF THE ACTIVITIES OF THE VENTROLATERAL ORBITO-FRONTAL AREA IN THE RAT

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Abstract—Clinical and recent imaging reports demonstrate the involvement of various cerebral prefrontal areas in the processing of pain. This has received further confirmation from animal experimentation showing an alteration of the threshold of acute nociceptive reflexes by various manipulations in the orbito-frontal cortical areas. The present study investigates the possible involvement of this area in the modulation of neuropathic manifestations in awake rats. Several groups of rats were subjected to mononeuropathy following the spared nerve injury model, known to produce evident tactile and cold allodynia and heat hyperalgesia. The activity of the ventrolateral orbital areas was selectively blocked by using either chronic or acute injection of lidocaine, electrolytic lesion, or chemical lesion with kainic acid or 6-hydroxydopamine (6-OHDA). The effects of these manipulations were compared with those following lesion of the somatic sensorimotor cortical areas. Local injection of lidocaine resulted in a reversible depression of all neuropathic manifestations while electrolytic or chemical lesions elicited transient attenuation affecting mainly the heat hyperalgesia and to a lesser extent the cold allodynia. The magnitude of the observed effects with the different procedures used can be ranked as follows: 6-OHDA<lesion<electrolytic lesion<kainic acid lesion<lidocaine injection. The observed effects were transient despite the permanence of the lesions while lesion of the somatosensorimotor cortices produced sustained reduction of the neuropathic manifestations. Our results correlate well with the established connections of the ventrolateral orbital area with the thalamic nucleus subnucleus involved in the procession of thermal nociception. The transient effects reported following permanent lesions in the orbital areas may reflect its flexible role in pain modulation. This observation provides further evidence on the plasticity of the neural networks involved in the regulation of nociceptive behavior.

Key words: wononeuropathy, allodynia, hyperalgesia, nociceptive behavior, somatic sensorimotor cortex, thermal nociception.

The role of the cerebral cortex in pain processing and perception has been clearly established during the last decade following the advent of the non-invasive human brain imaging techniques. Human cortical activation by various nociceptive stimuli or in some clinical situations has been described in several reports (Coghill et al., 1994; Derbyshire et al., 1997; and for review see Apkarian, 1995; Kenshalo and Douglas, 1996; Casey, 1999; Treede et al., 1999, 2000). Most of the observations on the human brain, which were reproduced in animal experimentation, have shown multiple cerebral representations of nociceptive information. Moreover, bilateral activation has been observed in various cerebral areas, especially in the prefrontal cortex (Kenshalo and Douglas, 1996).

The orbital area is a main component of the prefrontal cortex comprising the orbital surface of the frontal lobe (Elliott et al., 2000; Ongür and Price, 2000). Surgical lesion of the orbital cortex has been shown in patients to provide relief from chronic pain (Grantham, 1951). A revival of interest in the role of this area has been triggered by the demonstration of reciprocal connections between the ventral-lateral orbital (VLO) area and the thalamic nucleus submedius (Sm) (Craig et al., 1982). The latter nucleus is known to receive significant input from the spinal lamina I neurons which are activated by nociceptive stimuli (Craig and Burton, 1981; and for review see Willis, 1997).

Early research work on experimental animals has suggested a possible involvement of the orbito-frontal area in the modulation of nociceptive behavior. As illustration, ablation of this area in cats (Reshetniak and Kukushkin, 1989) or its block by local injection of lidocaine in rats (Cooper, 1975) has been reported to increase the thresholds of nociceptive reflexes. Reciprocally, noxious stimuli have been shown to increase the blood flow in the orbito-frontal cortex of the cat (Tsubokawa et al., 1981).

More recent research, using single cell recording and selective activation or blocking methods in experimental animals, has led to the following main findings. First, the activities of orbito-frontal neurons are altered by somatic and visceral noxious stimuli (Snow et al., 1992; Follett and

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Abbreviations: KA, kainic acid; 6-OHDA, 6-hydroxydopamine; PAG, periaqueductal gray; PWD, paw withdrawal duration; Sm, submedius; SNI, spared nerve injury; VLO, ventral-lateral orbital; VO, ventral orbital; 6-OHDA, 6-hydrooxydopamine.

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Dirks, 1995) and are depressed by morphine (Yang and Follett, 1998). Second, activated neurons have large and bilateral peripheral receptive fields (Backonja and Miletic, 1991) that show increased after-discharge in mononeuropathic rats (Backonja et al., 1994). Third, the activation of VLO can influence the firing of neurons in the brainstem pain-modulating centers (periaqueductal gray [PAG] and rostral ventro-medial medulla; Hutchison et al., 1996). This activation has been suggested as a substrate for the antinociceptive action of VLO activation by either morphine (Huang et al., 2001) or glutamate (Zhang S et al., 1997) injection or by electrical stimulation (Zhang Y et al., 1997; Zhang et al., 1998).

In summary, information from the various studies, based on either functional imaging of the human brain or observations in experimental animals, strongly suggests the involvement of the orbital area in the processing of nociception. Opposing views about the functional significance of this involvement have been suggested from animal experimental studies. Some reports attribute a pronociceptive role to the orbital cortex as reflected by the activation of its neurons by nociceptive stimuli and by the activation of on-cells in brainstem pain-modulating centers (Backonja and Miletic, 1991; Backonja et al., 1994; Snow et al., 1992; Follett and Dirks, 1995; Yang and Follett, 1998; Hutchison et al., 1996) and by the increased nociceptive thresholds following lesion or selective blocking of this area (Cooper, 1975; Reshetniak and Kukushkin, 1988, 1989). Other reports, however, tend to assign an antinociceptive role to the orbital area as revealed by the increased latencies of nociceptive reflexes due to direct activation of this area (Zhang et al., 1998; Huang et al., 2001) or through the indirect activation of brainstem pain-modulating centers (Zhang S et al., 1997; Zhang Y et al., 1997).

With few exceptions, all the reported observations have been based on acute nociceptive tests and performed on animals under anesthesia. Both situations cannot be considered optimal for the exploration of the cerebral networks involved in nociception. In the present study, we propose to investigate the role of the ventral orbital (VO) and VLO areas in neuropathic manifestations. This study was carried on awake rats subjected to mononeuropathy using the spared nerve injury (SNI) model described by Decosterd and Woolf (2000). Due to the established reciprocal connection between these orbital areas and the thalamic nucleus Sm, special attention was focused on the mechanical and thermal manifestations of neuropathy.

EXPERIMENTAL PROCEDURES

General procedures

Adult Sprague–Dawley female rats weighing 250–350 g were used in all the experiments. The rats were housed under standard colony conditions (T° 22 ±2 °C; 12-h dark/light cycle, with free access to food and water) and all the tests were performed during the light part of the cycle.

All surgical procedures were performed under deep anesthesia with i.p. injection of ketamine (Ketalar 40 mg/kg) preceded by preanesthesia with i.p. injections of atropine (0.05 mg/kg) and chlorpromazine (8 mg/kg). All the procedures were conducted with strict adherence to the guidelines for pain experimentation on awake animals (Zimmermann, 1983) and were approved by the Institutional Animal Care Committee. Experimental groups were planned to contain the minimum number of animals (5 to 6 each) needed to obtain statistical significance.

All the rats were subjected, under deep anesthesia, to surgical isolation of the three main divisions of the sciatic nerve in the left leg at the level of the popliteal fossa. The posterior tibial and the common peroneal nerves were ligated and cut, leaving a gap of few millimeters between the two separated ends. Special care was taken to keep the sural nerve intact, and the wound was closed and sutured in layers. At the end of surgery, each rat received one prophylactic injection of penicillin (1 million IU, i.p.) and was allowed 3–4 days for recovery.

Experimental groups

The results of this study are based on observations from seven groups of rats (n=5–6 each) all subjected to SNI mononeuropathy in their left legs. Individual rats received one of the following treatments to their brain area, as one group per treatment.

Selective blocking of the orbital area was performed on the first two groups of rats through either chronic infusion or acute injection of lidocaine 2%. Electrolytic lesion of the orbital area was performed on the third group and destruction of either dopaminergic terminals or neuronal cell bodies of this area was performed on another three groups of rats. Surgical removal of the somatosensorimotor cortical areas was carried out on the seventh group of rats.

Procedures for blocking or lesioning of the orbital areas

Several procedures were used to produce acute or chronic alteration of the functions of the VO and VLO areas. Chronic blocking of the neuronal activities was achieved with the use of miniosmotic pumps (ALZET, model 2002, 0.5 μl/h; Alza Pharmaceuticals, Palo Alto, USA). Under deep anesthesia, a small hole was drilled in the exposed skull overlying the VLO area. The brain infusion cannula was introduced according to the following stereotaxic coordinates: antero-posterior, 3.7–4; lateral, 1–1.5; vertical, 4–4.5 mm (Paxinos and Watson, 1986). After fixation, the cannula was connected through a catheter to the pump reservoir implanted under the skin of the abdominal area. In a second group of rats, similar coordinates were used for the implantation of a guide cannula (Plastic One, Inc., VA, USA) that was used for the injection of 0.5–1 μl of lidocaine for acute anesthetic block in another group of rats.

Unilateral electrolytic lesion of the VLO was performed with bipolar coaxial electrodes (David Kopff Instrumentations [DKI] 10-NE.100) introduced stereotaxically at the same coordinates used for the first two groups. A constant current (20–30 μA) was applied between the two electrodes for 30 s. The current parameters and the electrodes used have been shown previously to produce evident lesions in various thalamic nuclei (Saadé et al., 1999).

Selective chemical lesions were achieved by injecting kainic acid (KA; Sigma) or 6-hydroxydopamine (6-OHDA; Sigma) using a Hamilton syringe (5 μl) fixed on a DKI injector (Model 5000, USA) and the needle tip was placed stereotaxically in the VLO area. For specific excitotoxic lesion of neuronal cell bodies, each rat received 0.5–0.75 μl of KA (0.01 M) dissolved in saline. This volume was injected over a period of 2 min (Saadé et al., 1997). For lesion of the dopaminergic terminals in the VLO, each rat received 3 μl of 6-OHDA (6 μg/μl) dissolved in 0.1% ascorbic acid (Saadé et al., 1997). This volume was injected over a period of 5 min. All rats subjected to chemical or electrolytic lesions in the VLO, received one dexamethasone injection (2 mg/kg, s.c.) at the end of the surgical procedure.

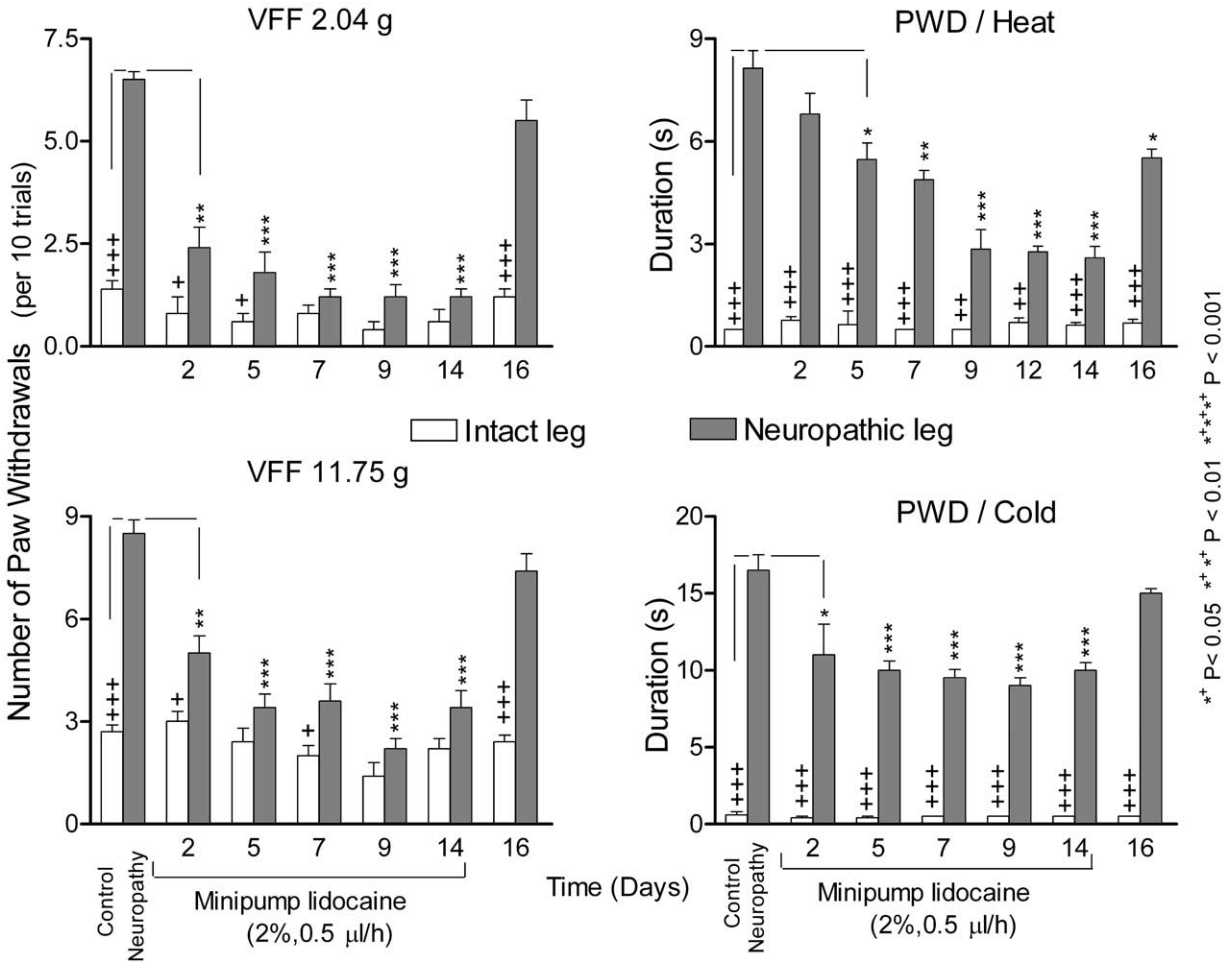

Fig. 1. Sustained attenuation of neuropathic manifestations in a group of rats ($n=6$) subjected to microperfusion of the VLO area with lidocaine 2%. ALZET miniosmotic pumps (0.5 μl/h; 2 weeks) were used to perfuse the orbital area through chronic cannulae stereotaxically placed in the rats' brains. Each bar in each panel represents the mean±S.E.M. of the measurements made on all animals at the indicated time interval for each test. The significance of difference was measured with reference to control neuropathy (*) before the injection or with reference to measurements made on the intact (contralateral) leg at the same time interval (+).

Surgical lesion of the somatic sensorimotor cortical areas

The head of each anesthetized rat was fixed in the stereotaxic frame and the skull covering the right cerebral hemisphere was opened at the following coordinates: antero-posterior between +3 and −4 mm and lateral between 1.5 and 4.4 mm (Paxinos and Watson, 1986). After sectioning the dura, the somatic sensory and motor cortical areas were removed by gentle suction though a 1.5 mm diameter glass pipette as described by Wall et al. (1988). At the end of surgery, the lesioned cortical area was covered by gelfoam and the scalp was sutured. Each rat received daily injection of dexamethasone (2 mg/kg, s.c.) for a period of 3 days. One week after surgery, all the rats showed normal recovery without notable signs of paralysis and gained weight normally.

Behavioral tests

Two procedures were used to test the hyperreactivity of the rats to innocuous mechanical and cold stimuli. For the mechanical (tactile) allodynia, the rats were placed in individual compartments of a cage with a floor made of a wire grid to allow free access for stimulation of the plantar area. Von Frey filaments of the two calibers 2.041 g (18.5 mN) and 11.749 g (106.7 mN) were used. Each hair was applied to the lateral aspect of the plantar surface of the paw with an upward force just enough to bend the filament. The number of times the rat withdrew its leg out of 10 trials was recorded. Consecutive hair stimulations to the leg were applied at a minimum interval of 3 s. Both hairs produced less than three responses in normal rats and more than five responses (per 10 trials) in the neuropathic leg (El-Khoury et al., 2002).

Cold allodynia was assessed by the method of Choi et al. (1994). A 50 μl solution of acetone was dropped on the lateral surface of the paw and the duration of the withdrawal reaction was measured. This procedure did not produce withdrawal reaction in the intact leg but elicited a sustained reaction in the neuropathic leg. One test was performed on each leg during a test session. Minimal and maximal values of 0.5 s and 20 s were assigned to a test eliciting no reaction or maximal reaction, respectively.

The increased reaction to noxious heat stimuli (hyperalgesia) was measured by the paw withdrawal duration (PWD) test. It is well established that the SNI does not produce significant alteration of the PW latency when compared with the intact leg, but leads to a significant increase in the duration of the reaction (Decosterd and Woolf, 2000). A heating spot of light was oriented

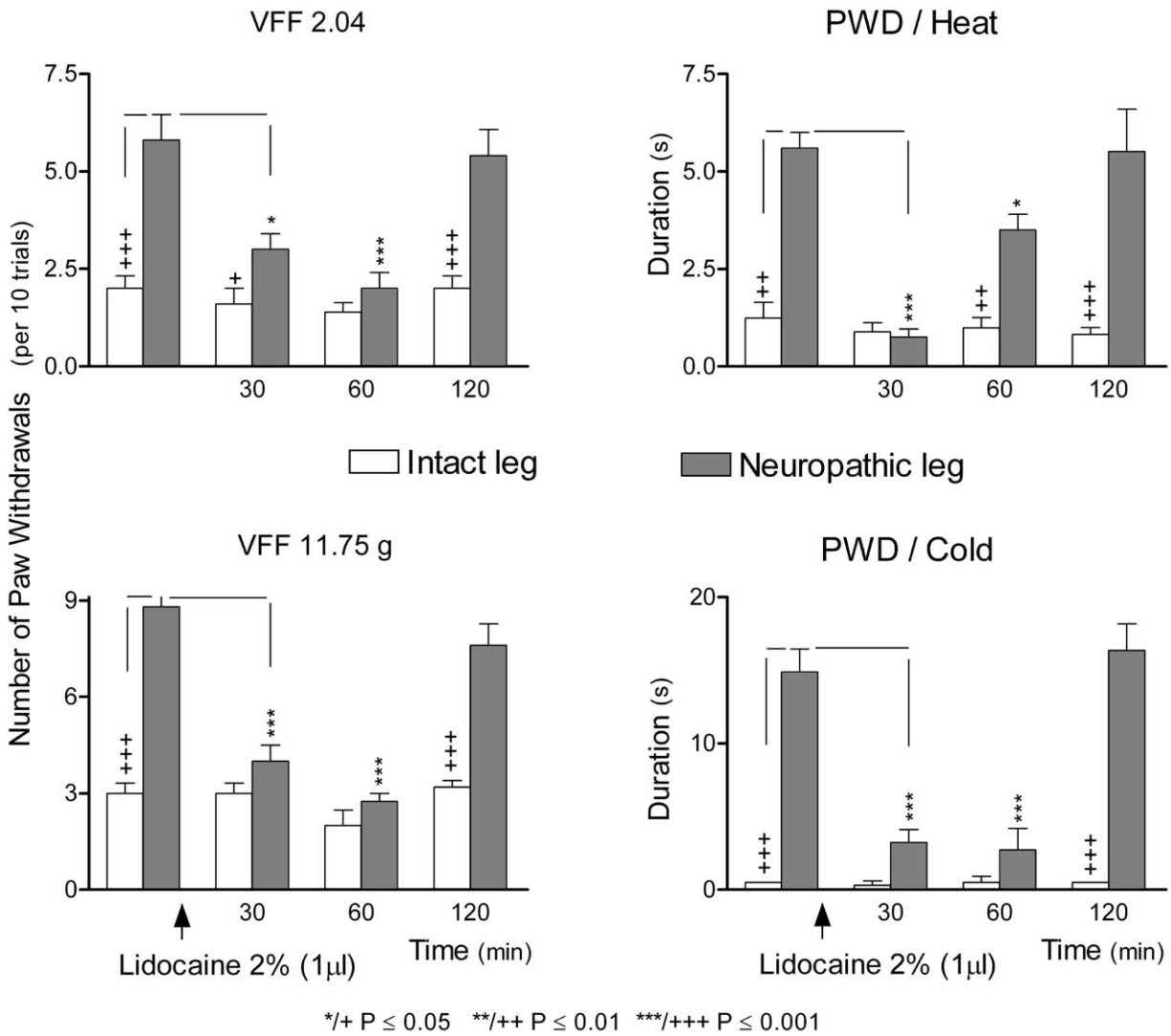

Fig. 2. Time course of the effects of microinjection of lidocaine (2%, 1 μl) in the VLO area on the neuropathic manifestations in a group of rats ($n=6$). All neuropathic manifestations (allodynia and hyperalgesia) were equally and reversibly reduced by lidocaine injection.

to the plantar surface of the leg to elicit a withdrawal reaction after an average latency of 7–8 s. A minimal arbitrary time of 0.5 s was assigned to a brisk reaction of the intact leg and a maximum cutoff time of 10 s was assigned to a sustained withdrawal reaction elicited by the neuropathic leg (El-Khoury et al., 2002). Each leg was subjected to two trials performed after a minimum interval of 2 min. The average of the two values was reported as the PWD for each rat.

The behavioral tests were performed two to three times per week. A random order was followed in the execution of the battery of tests.

Histology

At the end of the observation period, each rat was deeply anesthetized (75 mg/kg, pentobarbital, i.p.) and transcardially perfused with formalin (10%). The brains were isolated and processed for histological control of the placement of cannulae or lesions. For this purpose, serial sections (40 μm thickness) were stained with Nissl stain and screened under the microscope. Reported results are based on observations made on rats with accurate location of the injections or lesions in the ventral lateral and/or VO areas.

Data analysis

Neuropathic manifestations (allodynia and hyperalgesia) were assessed in rats with reference to observations made on the same rats before the induction of mononeuropathy and also by comparing the measurements made on the neuropathic leg with reference to those made on the contralateral intact leg.

For the assessment of allodynia (tactile or cold) and hyperalgesia, all measurements made on rats in an experimental group were averaged and expressed as mean±S.E.M. for a certain period of time.

Basal levels of neuropathic manifestations were measured before injection or lesion in VLO, and compared with measurements made at different times following the performance of a particular procedure. Comparison between measured values for each type of test were made using ANOVA followed by Tukey post hoc test. Statistical operations and graphics were made using a Prism 3 GraphPad package (GraphPad, Software, Inc., CA, USA).

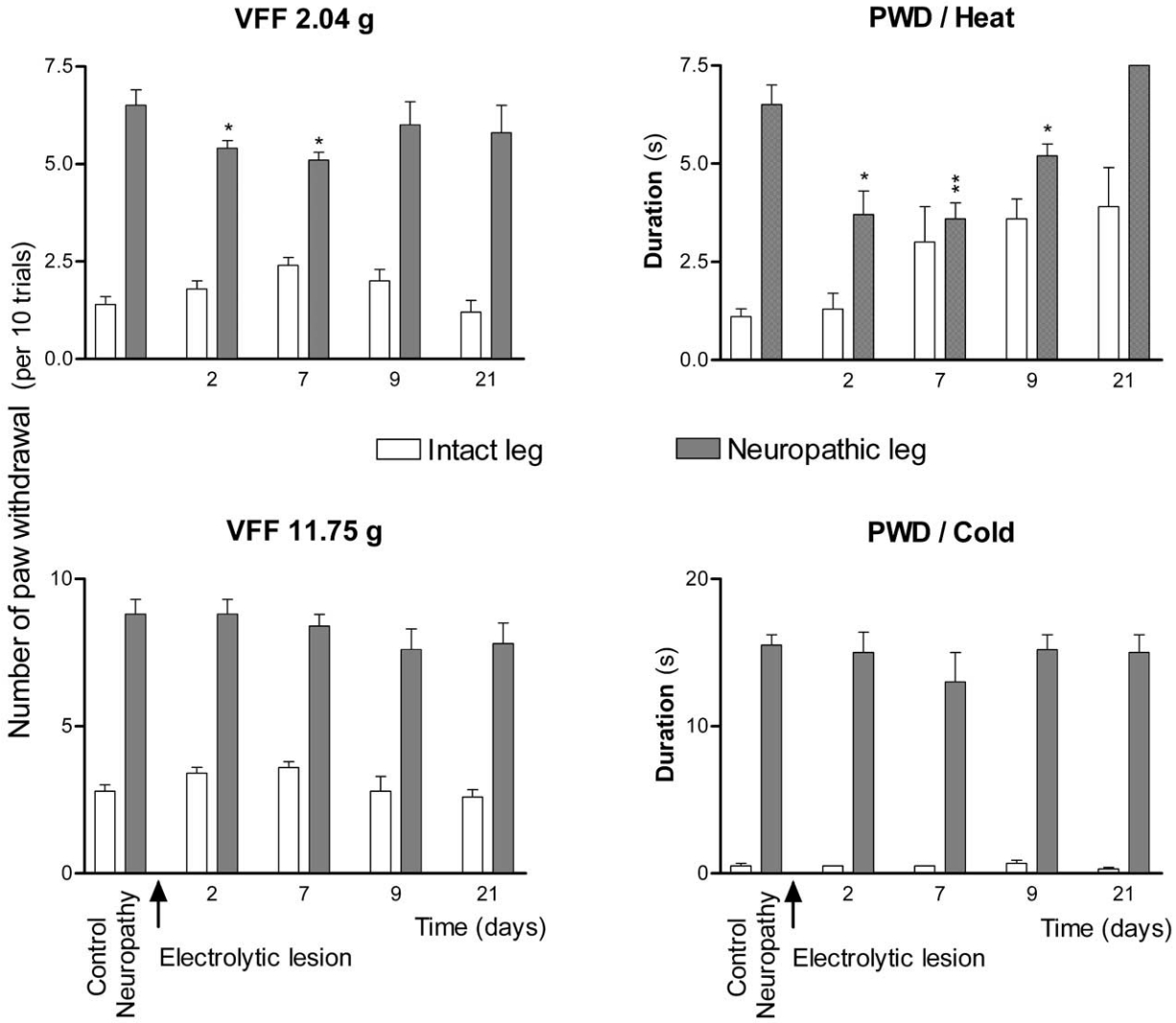

Fig. 3. Time course of the effects of electrolytic lesion placed in the ventrolateral orbital area on the neuropathic manifestations in a group of six rats. Lesions were performed in the contralateral orbital area, with reference to the neuropathic leg. Moderate effects were observed on tactile allodynia and heat hyperalgesia.

RESULTS

Allodynia and hyperalgesia in the SNI model became evident during the first week, peaked during the second week and maintained a plateau over weeks or months following the induction of mononeuropathy (Decosterd and Woolf, 2000; El-Khoury et al., 2002). Therefore, procedures aiming to interfere with the function of the orbital area were performed on rats with established neuropathic manifestations, i.e. 2–3 weeks following the induction of neuropathy.

Individual rats in each group, with established allodynia and hyperalgesia, were subjected to the same type of procedure performed on their orbital areas and were killed at the end of the observation period.

Effects of lidocaine injection

Chronic perfusion of the VO–VLO areas with lidocaine 2%, at a rate of 0.5 μl/h, was performed on six rats over a period of 2 weeks. A significant reduction of all the neuropathic manifestations was observed with the first test after the surgical procedure for pump implantation. Fig. 1 shows a complete recovery from tactile allodynia by the end of the first week, whereby the reactivity to hair filament became equal in the neuropathic and intact legs; this depression was maintained until the end of the perfusion period. Recovery of tactile allodynia to the preinfusion level was complete 2 days after the end of perfusion. At the same time, significant reduction in the cold allodynia and heat hyperalgesia was observed during the 14 days of perfusion. However, the increased reactivity of the neuropathic leg was maintained at a level significantly higher than the intact leg.

Acute injection of lidocaine 2% (1 μl) in another group of rats produced comparable results to those observed with chronic perfusion, but the effects peaked at 30 min and disappeared within 2 h following the injection (Fig. 2).

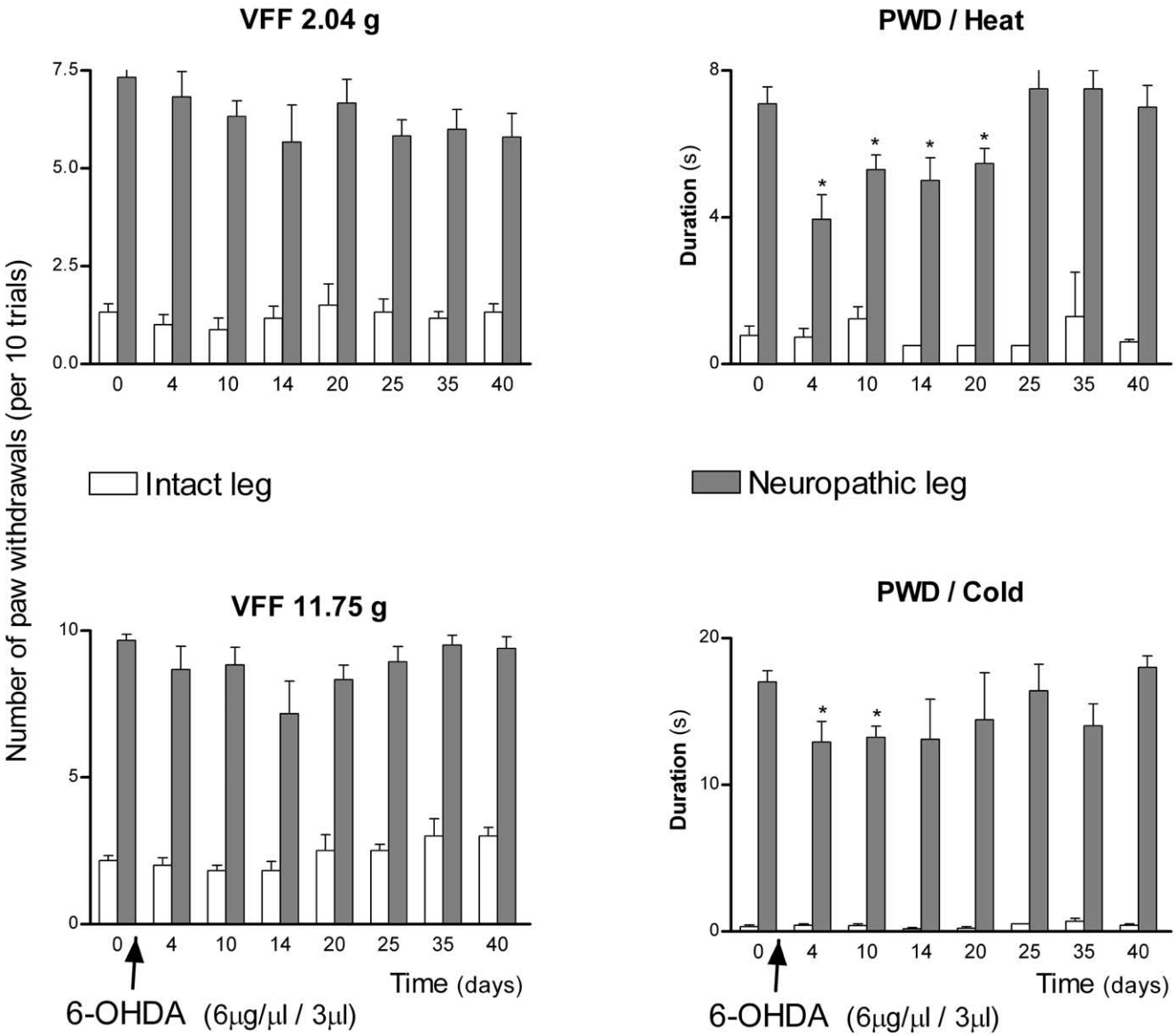

Fig. 4. Time course of the effects of 6-OHDA injection in the VO area on the neuropathic manifestations in a group of five rats. Lesions of dopaminergic terminals in the VLO area produced moderate and transient attenuation of heat and cold hyperalgesias. Tactile allodynia was not affected.

It is worth noting here that acute injection of lidocaine in either ipsilateral or contralateral VLO produced comparable effects on the neuropathic manifestations. Injection of 1 μl of sterile saline did not produce significant alteration in the neuropathic manifestations (data not shown).

Effects of selective lesions in the orbital area

Electrolytic lesions were placed in the VO-VLO area contralateral to the neuropathic legs of a group of rats. As shown in Fig. 3, this lesion resulted in moderate and short-lived attenuation of tactile allodynia. More pronounced attenuation of heat hyperalgesia was observed. Both effects, however, disappeared within 1 to 2 weeks. Cold allodynia did not show significant alteration (Fig. 3).

Microinjections of 3 μl of 6-OHDA (3 μg/μl), known to produce selective lesion of the dopaminergic terminals, resulted in a transient and moderate reduction of the heat hyperalgesia and the cold allodynia. Tactile allodynia did not show significant alteration (Fig. 4).

The effects of selective unilateral (contralateral to the neuropathic leg) or bilateral lesion of neurons in the VO–VOL areas by microinjection of KA (0.75 μl/0.01 M) are illustrated in Figs. 5 and 6, respectively. The most pronounced effects (reduction) of the contralateral lesion were observed on the heat hyperalgesia (Fig. 5). Bilateral lesions produced more pronounced effects on heat hyperalgesia and cold allodynia with a moderate and short-lived attenuation of the tactile allodynia (Fig. 6).

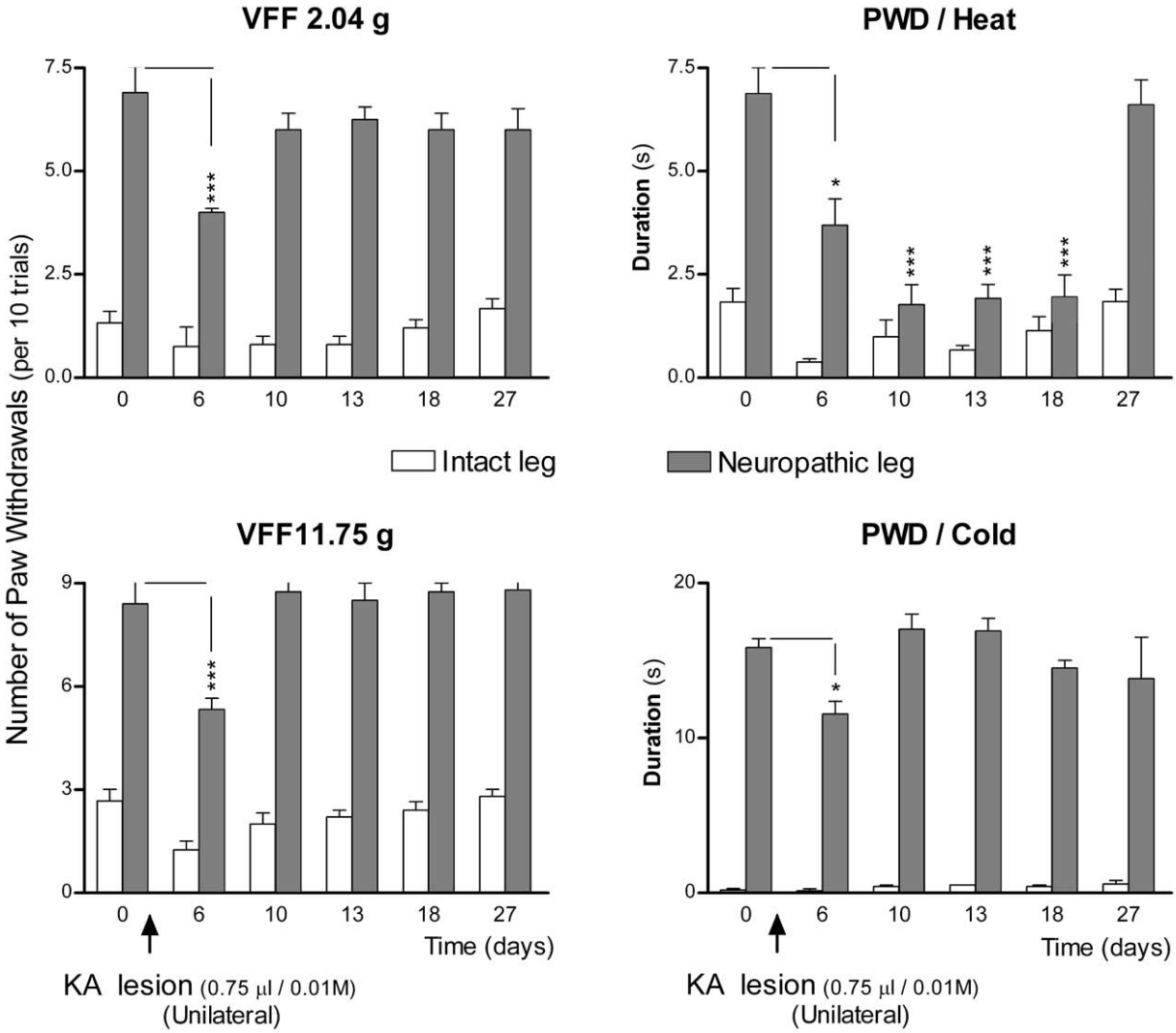

Fig. 5. Injection of KA in the VLO area, contralateral to the neuropathic leg, produced a short-lived attenuation of neuropathic manifestations, except for thermal hyperalgesia. Each bar represents the average of measurements performed on a group of five rats at the corresponding time interval. Rats received KA injection 2 weeks after the induction of neuropathy (Decosterd and Woolf, 2000), the time that corresponds to the full development of neuropathic manifestations.

All observed effects, however, disappeared with time and the neuropathic manifestations regained their original levels observed before injection.

Effects of lesion of the somatic sensorimotor cortex

Extensive lesion of the somatosensory and motor areas in the frontal and parietal cortex was performed on a separate group of rats. This lesion resulted in significant attenuation of all the neuropathic manifestations (Fig. 7).

Tactile allodynia disappeared completely within 2 weeks after the lesion, and the effect was maintained over a period of 10 weeks. Heat hyperalgesia and cold allodynia were less affected and the rats maintained a significant hyperresponsiveness to both tests during the observation period.

After 10 weeks following the cortical lesion, all rats of the same group were subjected to SNI mononeuropathy performed on the right leg (ipsilateral to the lesioned cerebral cortex). As shown in Fig. 7, tactile allodynia and heat hyperalgesia did not develop during 4 weeks after the induction of the second leg neuropathy. The only neuropathic manifestation that developed was the cold allodynia, but its level did not reach that observed in neuropathic rats before the cortical lesion (Fig. 7).

DISCUSSION

This study reports on the effects of various methods of blocking of the orbital cortical areas on the neuropathic manifestations in awake rats. Special care was taken in the design and execution of this study to fulfill two major requirements: first, to work on awake animals free of anesthesia and subjected to a strict minimum stress during the conduction of the tests; second, to select an

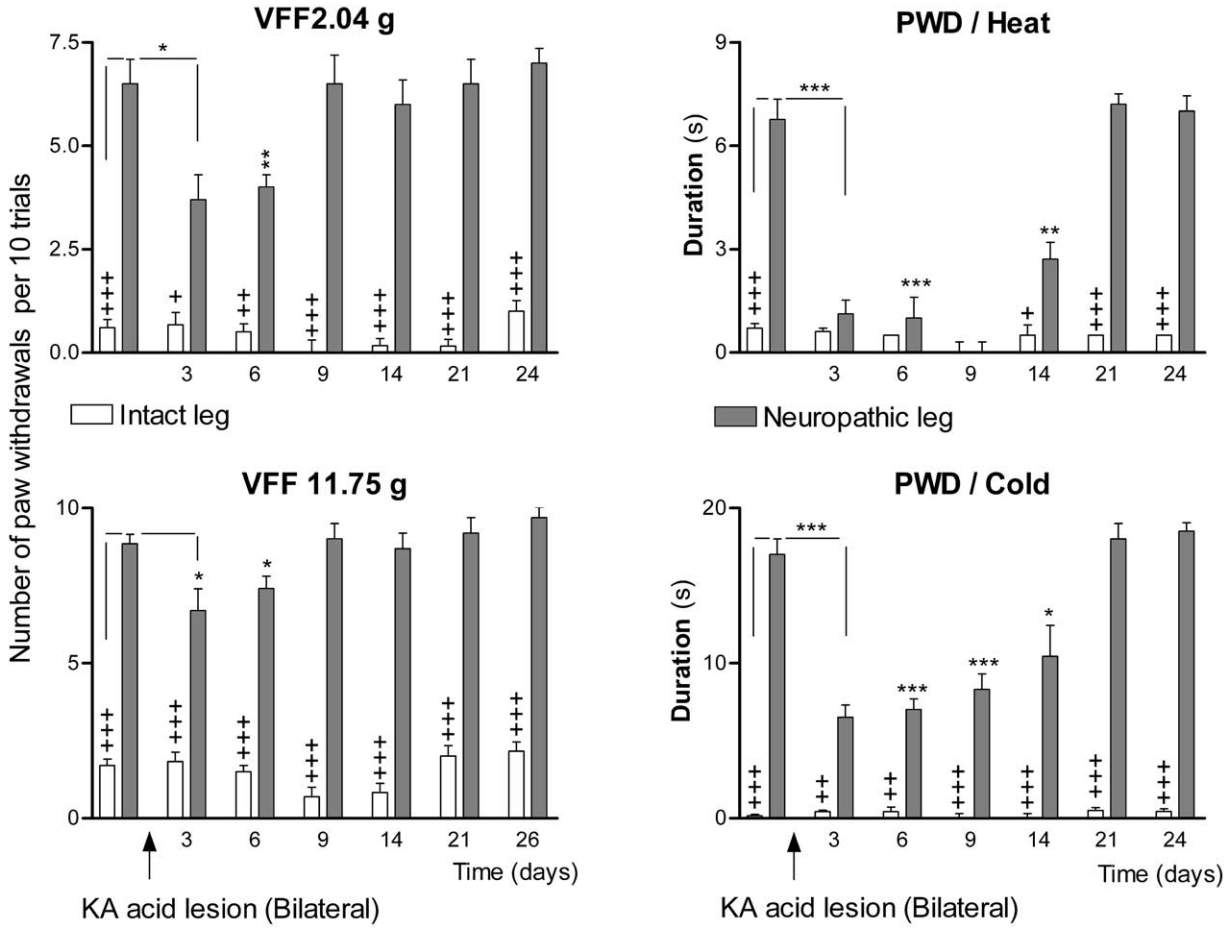

Fig. 6. Effects of bilateral injections of KA (0.75 μl, 0.01 M) in the VO areas on neuropathic manifestations in a group of rats (n=6). Bilateral lesions produced a relatively more pronounced attenuation of neuropathic manifestations especially cold allodynia and heat hyperalgesia.

experimental model simulating chronic nociception or pain.

The SNI model has been well characterized by its ability to produce stable neuropathic manifestations over a long period of time (Decosterd and Woolf, 2000; El-Khoury et al., 2002). Furthermore, the observed neuropathic manifestations included tests for mechanical and thermal allodynia and hyperalgesia, that are supposed to be processed by separate neuronal mechanisms at different levels of the peripheral and central nervous systems (Bester et al., 2000; Blomqvist and Craig, 2000; Saadé et al., 2002).

An important observation emanating from the present work is the reduction of the neuropathic manifestations by all the procedures involving partial or complete blocking of the activities of the orbital areas. This attenuation ranged from significant effects on allodynia and hyperalgesia following lidocaine injection to a mild and short-lived attenuation of the hyperalgesia with either electrolytic lesion or 6-OHDA injections. It is important to note here that, the effects were bilateral (as illustrated in the case of lidocaine injection), increased by bilateral treatment (as illustrated in the case of KA lesion) and were expressed preferentially on the heat hyperalgesia as assessed by the duration of the PWD. The tactile allodynia showed the least alteration in response to the different treatments.

Postmortem histological controls (Fig. 8) showed that all the injections and the lesions involved the VLO with moderate encroachment on the VO area in few cases. In all cases, however, the lesions or the injections did not involve the entire rostrocaudal extent of the VLO. Therefore, the reported effects cannot be considered as a result of a total blocking or lesion of the area. On the other hand, one may have to take into consideration the possible damage to the cortical area overlying the VLO (i.e. frontal cortex) due to the penetration of the injecting cannulae or electrodes. This possibility is of minor importance, since the basal levels of allodynia and hyperalgesia did not show significant alteration after the implantation of cannulae or with saline injection when compared with measurements made on the same rats before these procedures.

Two main observations from the present study appear to deserve further discussion. These are the variability of the effects of the different treatments on the different manifestations of neuropathy and the reversibility or recovery from the effects despite the persistence of the treatment or the lesion.

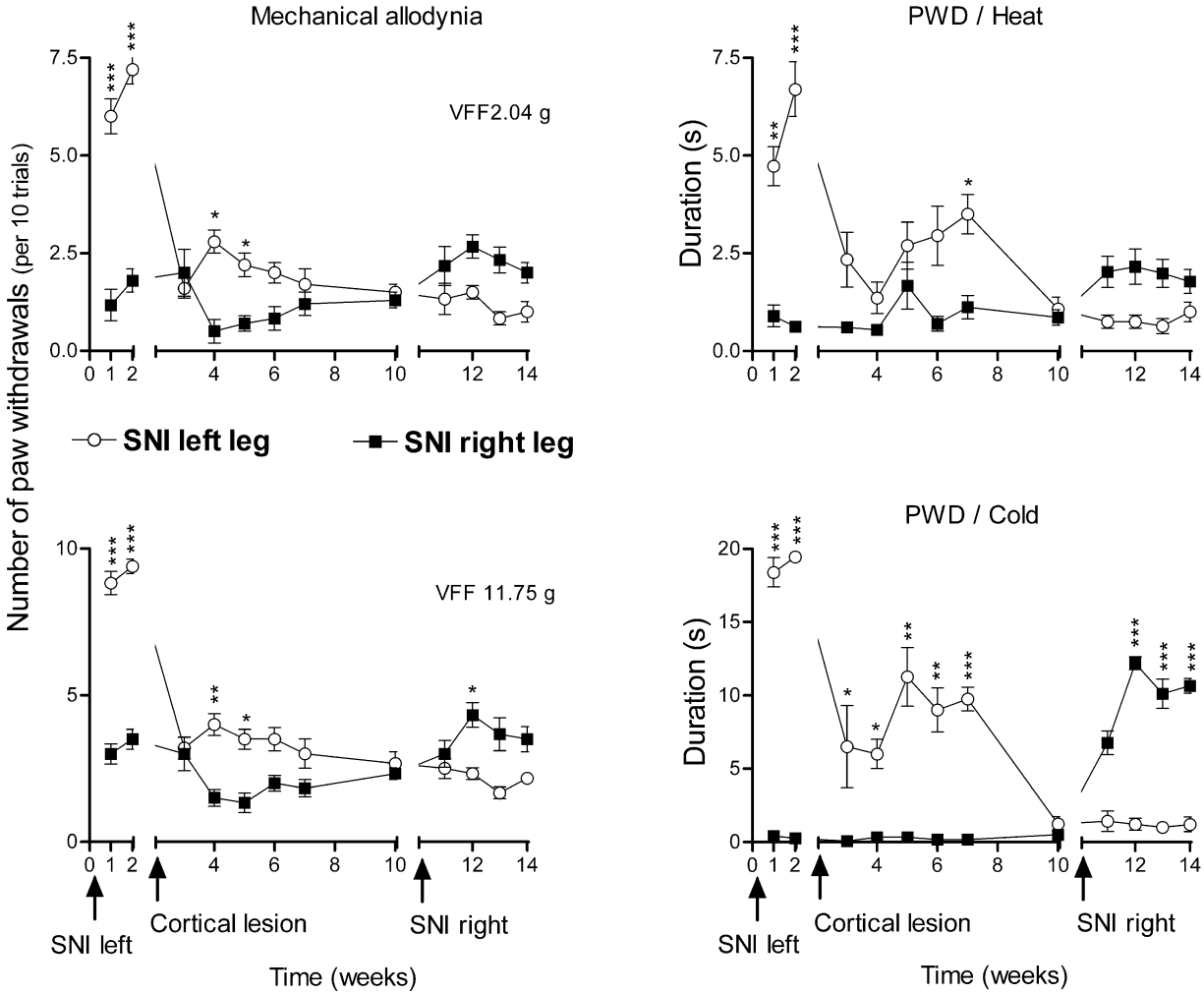

Fig. 7. Surgical removal of the somatic sensorimotor cerebral cortical areas abolished the neuropathic manifestations in a group of rats (n=6). Rats were first subjected to neuropathy in the left leg following the method of SNI. Surgical ablation of the somatic sensorimotor areas in the right cerebral cortex was performed 2 weeks after the induction of mononeuropathy. All neuropathic manifestations (except cold allodynia, which was significantly reduced) were abolished during a period of 10 weeks with no signs of recovery. Mononeuropathy was then induced in the right leg (ipsilateral to the lesioned cerebral cortex). The only neuropathic manifestations observed was the cold allodynia.

The design of the study was based on the assessment of tactile and cold allodynia and the heat hyperalgesia. For the tactile allodynia, we used two hairs at two mechanical forces ranging from mild or moderate touch (2.04 g) to moderate pressure (11.75 g). Both hairs can develop bending forces below the threshold of nociception (15 g; see Decosterd and Woolf, 2000; El-Khoury et al., 2002). This choice allowed the observation of a wide range of variations of tactile reactivity which is reflected by the development of tactile hyperreactivity from 2–3/10 trials to 6–10/10 trials after the development of neuropathy. However, the tests for cold allodynia or heat hyperalgesia did not allow a gradation of the intensity of stimulus similar to that used in tactile allodynia since for the former, the cold produced by acetone drop does not produce significant withdrawal reaction in the intact leg and produced hyperreactivity in the neuropathic leg. The same applies for the heat hyperalgesia with the difference that the stimulus used is in the nociceptive range, producing a phasic or brief withdrawal reaction in the intact leg while it produced a prolonged withdrawal with some flinching in the neuropathic leg.

Apart from the total blocking with lidocaine, all the procedures leading to partial blocking of the activities of the orbital area produced less pronounced and less persistent effects on tactile allodynia. The importance of the effects ranked in increasing order as follows: 6-OHDA lesion<electrolytic lesion<unilateral lesion with KA<bilateral lesion with KA. Thus, one can conclude that the dopaminergic terminals play a minimal role in the function of this area, while neuronal activities of this area play a major role in nociceptive modulation. Previous reports from our laboratory have revealed opposite effects of lesions of either dopaminergic terminals or neuronal cell bodies in the basal ganglia, on nociception. This was expressed by increased nociception following lesion of dopaminergic terminals and decreased nociception induced by lesion of neuronal cell bodies in

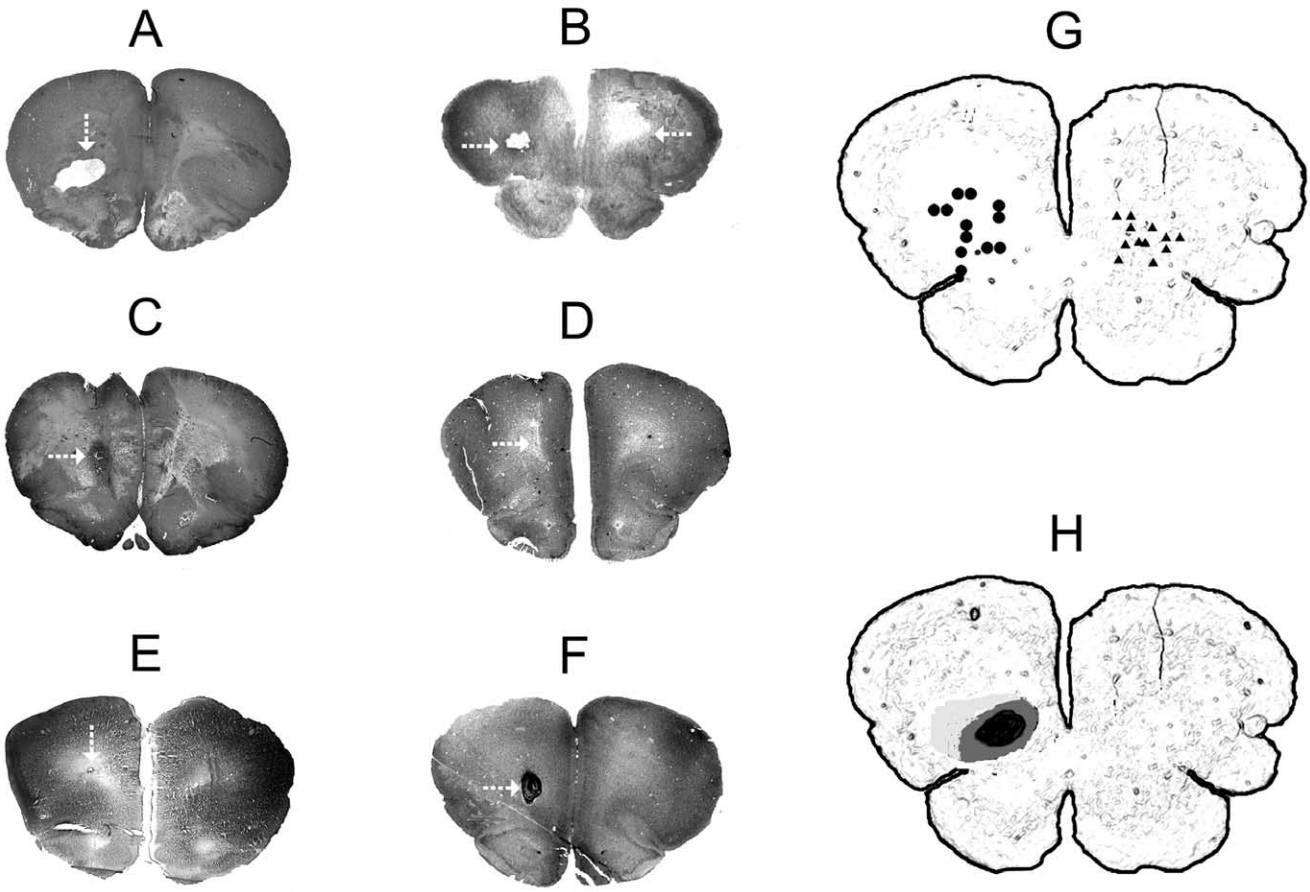

Fig. 8. Transverse sections across the orbito-frontal areas of the brain showing the locations of injections or lesions. A and B, microscopic views illustrating the location of lesions by either KA injection (A, unilateral; B, bilateral) or by electrolysis (C). The locations of the cannulae used for acute or chronic microinjections are illustrated in D and E, respectively. The extent of the diffusion of 1 μl of China ink is illustrated in F. All sites (A–F) are indicated by arrows. G and H, reconstructed drawings showing the locations of the tips of the injecting cannulae (triangles for the miniosmotic pumps and circles for the guide cannulae in G) and the location of the lesions (with shades from dark to light according to the extent of the lesion in H).

the striatum (Saadé et al., 1996, 1997). In the present study, both lesions decreased neuropathic manifestation with a minimal effect produced by the lesion of dopaminergic terminals. This discrepancy may be related to a difference in the connectivities between these two centers with other brain areas. Unlike the remaining parts of the prefrontal cortex, the VLO areas have reciprocal connection with the thalamic nucleus Sm (Craig et al., 1982; Coffield et al., 1992; Yoshida et al., 1992) and send efferents to the PAG and the lateral hypothalamus (Coffield et al., 1992; Reep et al., 1996; Floyd et al., 2000, 2001). Thus, the selective effects of VLO lesion on thermal neuropathic manifestation can be related to the special connectivity of this area to the nucleus Sm which is known to receive thermoceptive input from the spinal and trigeminal lamina I system (Craig and Dostrovsky, 1991; Willis, 1997).

Several clinical and experimental reports have shown long-lasting depression of neuropathic manifestation following either peripheral ganglionic block (for a review see Bonica and Buckley, 1990; Fields et al., 1999), central electrical stimulation (El-Khoury et al., 2002; and for a review see Simpson, 1999) or tract lesions (Bian et al., 1998; Miki et al., 2000; Ossipov et al., 2000). The attenuation of neuropathic manifestations by the various treatments of the VLO was transient and was followed by a recovery of the syndromes despite the persistence of the lesions. On the contrary, lesion of the somatic sensorimotor cortices produced long-lasting and bilateral attenuation of these manifestations with partial sparing of the cold allodynia. This discrepancy in the observed effects suggests that the VLO can contribute to the modulation of neuropathic pain without being a mandatory relay center. In contrast, the cortical somatosensory and motor areas constitute obligatory centers for the reception of allodynia and hyperalgesia with a possible dual representation of the cold hyperalgesia that can bypass the primary cortical areas through the thalamic nucleus Sm projecting to the orbital area (Craig and Burton, 1981).

The overall results of the present study are in line with the early reports on analgesia produced by orbito-frontal lesion or block (Grantham, 1951; Cooper, 1975; Reshetniak and Kukushkin, 1989) and on the activation of orbital neurons by somatic and visceral nociceptive stimuli (Backonja and Miletic, 1991; Snow et al., 1992). Furthermore, our observations correlate well with the results of recent

imaging studies of human brains with chronic pain that demonstrate significant activation of the prefrontal cortex including the orbital area (Hsieh et al., 1995; Apkarian et al., 2001; Grachev et al., 2002). The apparent discrepancies between our results and those reported by Zhang S et al. (1997, 1998) showing decreased nociception induced by orbital stimulation can be attributed to one or both of the following reasons: first, differences in the animal model used (acute nociception versus neuropathic model); second, differences in the experimental conditions (recording in anesthetized animals versus observations on freely moving unanesthetized rats).

In conclusion, the results of the present study provide evidence for the involvement of the orbital cortical area in the modulation of nociceptive behavior induced by mononeuropathy. The alteration of neuropathic manifestations by the various manipulations (acute or chronic block or lesions) provides further evidence on the wide distribution and the plasticity of the neural networks involved in nociceptive behavior.

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