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BLOCKAGE OF VIBRISSAE AFFERENTS: II FOOTSHOCK THRESHOLD INCREMENTS

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INTRODUCTION

We have reported (12) deficits in motor activity on open field and on a learned performance after vibrissal afferent blockade. Lack of tactile information from whiskers could not explain our data because trimming vibrissae at fur level did not produce any significant effect on motor behavior. Thus we thought that our data would support the "tonic hypothesis", i.e. the idea that afferent activity would help to maintain some kind of central activated condition, which underlies the awake or aroused state (2, 3, 8, 11).

One of the ways of testing this idea is to verify the losses of or diminished responses in different behavioral situations after cancelling the afferents being studied (3). Along this line, there are two reports of diminished agressive behavior produced by repeated footshocks after vibrissal pad anaesthesia (16, 17). As a possible explanation these authors proposed that, in this experimental situation, the rats lacked the tactual information needed for guiding a directed attack. However, decrements in rat aggressive responding were not so clear after vibrissae triming (16). On the other hand, preliminary observations at our lab (6) showed diminished responses to nociceptive stimuli applied to either tail, hind or forefoot after vibrissal anaesthesia but not after trimming these tactile hairs. In accordance with results shown previously (12), lack of tactual information could not satisfactorily explain these changes. Since Thor and Giselli's (16, 17) studies dealt with rather complex behaviors, the present work studies the effects of vibrissal afferent blockade on a much simpler response: thresholds for a flexor response. These results were partially published elsewere in abstract form (6).

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Each rat was tested individually. A test session consisted in *a)* Two minutes of habituation, and *b)* a series of footshocks, according to the rule of an increase in pulse intensity after non-response and a decrease in pulse intensity after response (the up-and down method for small samples, see references 5 and 7). The series began with a shock intensity as close as possible to the flinch threshold for the treatment being observed, and was defined by preliminary observations for the seven groups studied. Pulses continued until a change in behavior occurred -nonresponse to response or *viceversa*- and finished four trials after the first change. In all cases, only two subsequent series were carried out on the same rat; analysis of the results was based on means of these two tests.

We measured the current threshold value for flinch responses, defined as the elevation of only one paw, during or immediatly after the shock, involving withdrawal from the floor. Thresholds were estimated as the "median effective intensity" according to procedures and formulas provided by Dixon (7).

RESULTS

a) *Effectiveness of the afferent blockage*

The effectiveness of both vibrissal blockage (either nerve section or reversible blockage) was routinely assessed by the absence of response to either strong blunt or sharp pinch on the vibrissal pad. Also in both procaine and physiological solution injected groups the spontaneous vibrissal movements were completely absent.

We have shown previously a generalized behavioral depression and hypomotility following bilateral vibrissal pad anaesthesia (12). Before the present experiments we did an unilateral injection with either procaine solution (1 cc, 0.5%) or saline (1.5-2 cc) in a few animals (not these used for threshold measures). These animals showed typical circling behavior (14), but not the generalized depressive effects. Thus the efficacy of the local anaesthesia (either chemical or mechanical) was tested by the absence of response to a strong pinch to the injected pad. General sensitivity outside the injected pad (including contralateral pad) was intact, and the lack of vibrissal movements was restricted to the injected side.

The nonchemical (*i.e.* mechanical) nerve blockage technique we chose is based on certain old neurphysiological and clinical observations: nerve conduction blocks can be produced by either mechanical compression and/or by ischaemia. The effects of compressing peripheral nerves has been extensively described (see ref. 15 for a review). As early as 1885, Halsted described the analgesic effect of saline injection, and Maskowicz in 1901 started using the pressure injection of procaine solution for analgesia (9). In 1927 Wischnewsky described the use of massive infiltration of weak procaine solutions (0.25%) to induce local anaesthesia, where the anaesthesia is induced more by the pressure than by the procaine (9). Our mechanical blockage of the vibrissal system can be explained as follows. The forced intradermal injection of physiological solution into vibrissal pads produced an obvious swelling and the "orange skin like" appearance, well known to be induced by this procedure (9). Since the vibrissal pad can be considered erectile tissue (10) the physiological solution injected should diffuse little into the sorrounding erectile tissue, producing mechanical pressure on the tranversing nerve fibers, which eventually results in a nerve conduction block. As expected, the

Crocker and Russell (4) in normal conditions (mean = 0.16 mAmp, standard error = 0.02). We did not find threshold differences between males and females, thus results are shown without sex differentiation.

Effects of different treatments on flinch responses are depicted in Figure 1, as means plus one standard deviation. Pulse intensities used in normal and control conditions ranged from 0.07 to 0.18 mAmp; from 0.15 to 0.38 mAmp for the IO nerve section group; from 0.18 to 0.91 mAmp for the procaine injected group and from 0,13 to 0.44 mAmp in the physiological solution injected group. With these pulse intensities we never observed any behavioral signs of pain (such as vocalizations or jumping).

Results of different treatments were not normally distributed; thus a common log transform was used for statistical analysis. A one way ANOVA was carried out for test significance of these results. Multiple comparisons vs. normal group (Bonferroni t-test) were used as a post-hoc test. Both mathematical and statistical procedures were performed using *SigmaStat* statistical software (V.2.0; SPSS Inc).

As is seen in Figure 1, the three groups with vibrissal afferent blockades (nerve section, procaine and physiological solution injected) needed larger values of current to obtain a flinch response than those required for the normal group. Statistical analysis shows that the groups were significantly different: $F(7,67) = 28.6$; $p < 0.001$. After injection of both procaine and physiological solution into the vibrissal pads, the current needed for flinch responses was significantly greater than for normal ($t = 10.25$; $p < 0.05$ and $t = 3.80$, $p < 0.05$ respectivelly). Pulse intensity values for flinch responses in the permanent blockade group were also significantly higher than in normal group ($t = 6.75$; $p < 0.05$). Vibrissae trimming and facial nerve section did not produce significant increments in the current needed for a flinch response. Finally, no significant differences were found between normal, sham and control anaesthesia groups.

It is interesting to note here that the mean pulse intensity needed for a flinch response in the transitory blockade group was twice the intensity reported by Croker and Russell (5) for jump responses in normal rats. A jump response was defined as rapid movements of three or more paws, involving a withdrawal from the floor. Moreover, the current needed for a flinch response after vibrissal pad anaesthesia (0.86 ± 0,02 mAmp) was similar to the increased flinch values reported by Croker and Russel (5) after subcutaneous morphine (26 mmol/Kg) injections (0,83 ± 0.02 mAmp). As we have already noted, only flinch responses were investigated in our studies. Thus there was no possibility of pain being inflicted in these experiments.

DISCUSSION

Clearly, our experimental results showed a decrease in footshock responses after vibrissal afferent blockade, in agreement with Thor and Giselli (16, 17). They postulated that the decreases in irritable fighting observed by them after vibrissal anaesthesia, were due to the lack of tactile information needed for guiding a directed attack. On the other hand, our results, for similiar experimental conditions, indicate

rissal deprivation was also reported by Watson (21) and Richardson (13). In our case it should be considered that responses in the infraorbital nerve sectioned group were measured at least 24 hours after surgery. The greater effect of transitory blockade was perhaps due to the the acute aspect of this deafferentation –i.e., in this case we were testing the rat without allowing any time for adapting to the new situation as in the other group. In this regard, we think that vibrissal local anaesthesia is an interesting way to assess acute effects of peripheral sensory deprivation because it can be carried out without any additional manipulation (i.e. without general anaesthesia).

With regard to this point it is possible that the effects of IO nerve section are more severe in the first hours after surgery. Obviously it is very difficult to clearly separate the effects of barbiturirate anaesthesia from the effects due to deafferentation itself at this time. However, we have invariably observed that general anaesthesia recovery (flexion, pupilary and righting reflexes) takes considerably more time (2-3 hours) than the same recovery after sham surgery. Although this fact may be of some interest, the detailed study of the time course of recovery from bilateral vibrissal pad deafferentation is beyond the scope of the present work.

The functional recovery after nervous injury and its dependence on factors such as age and lesion size are well known phenomena. Furthermore the compensatory mechanisms (i.e. funtional reorganization and/or structural changes) have been extensively studied (20). It may be of some importance to note that this capacity that allows the nervous system to compensate the deficits produced by injury would not argue against the functional importance of the lesioned area. As a classical expample the work of Adametz (1) could be quoted. He completelly destroyed the Mesencephalic Reticular Formation in many steps with sufficient time inbetween to allow for complete recovery. The final lesion of the whole reticular formation did not result in a state of coma. However, these well known results were never used to argue against the importance of the reticular formation in the maintenance of wakefullness. In our case, with the lesser behavioral effects of IO nerve section than vibrissal pad local anaesthesia, the extent of deafferentation should be considered in addition to time. Altough important, the bilateral blockage of vibrissal afferents is restricted and would silence only about 30% of the whole rat primary somatosensory cortex (22). Thus the parcial recovery in a relatively short time (24-48 hours) is not surprinsing considering that 70% of afferent somatosensory activity was left intact.

In summary we found significant increments of threshold to footshock after vibrissal afferent suppression. Lack of the specific tactual information provided by the vibrissae could not explain the diminished responsiveness to stimuli applied to the feet. We have shown that cutting the whiskers at fur level did not produce threshold increments. Summing up the present and the previous paper, we have found motor and sensory depressive effects of vibrissal afferent blockage that could not be completely explained by lack of the tactual information normally provided by the whiskers. Instead our data could be considered as evidence supporting the idea of a general tonic excitatory role for sensory inputs.

14. STEINER, H., WEILER, H.T. AND HOUSTON, J.P. Asymetries in crossed and uncrossed nigrostriatal projections dependent on duration of unilateral removal of vibrissae in rats. *Exp. Brain Res.*, **77**: 421-424, 1989.
15. SUNDERLAND, S. *Nerves and Nerve Injuries* (Second Edition). Churchill Livingstone, Pitman Press, London, pp. 70-73, 1978.
16. THOR, D.H. AND GISELLI, W.B. Prolonged supression of irritable aggression in rats by facial anesthesia. *Psychol. Rep.*, **33**: 815-820, 1973.
17. THOR, D.H. AND GISELLI, W.B. Vibrissal anesthesia and supression of irritable fighting in rats: A temporary duration effect in experienced fighters. *Physiol. Psychol.*, **3**: 1-3, 1975.
18. UEXKÜLL, J. VON. Zur Muskel und Nervenphysiologie des *Sipunculus nudus*. *Z. Biol.*, **40**: 269-344, 1896.
19. VINCENT, B.S. The Function of vibrissae in behavior of white rat. *Behav. Monogr.*, **1**: 7-81,1912.
20. WALL, J.T., XU, J. AND WANG, X. Human brain plasticity: an emerging view of the multiple substrates and mechanisms that cause cortical changes and related sensory dysfunctions after injuries of sensory inputs from the body. *Brain Res. Rev.*, **39**: 181-215; 2002
21. WATSON, J.B. Kinaesthetic and organic sensation. *Psychol. Rev. Monogr.* Suppl., **8**: 7-76, 1907.
22. WELKER, C. Microelectric delineation of fine grain somatotopic organization of SmI cerebral neocortex in albino rat. *Brain Res.*, **26**: 259-275, 1971.

SUMMARY

Because of their dense innervation rat vibrissae have been regarded as a very important sensory system. Many behavioral deficits have been reported by other authors after rat vibrissal afferent blockades. In the present work we found significant threshold increments to footshock following either reversible nerve block (procaine or nerve pressure) or section of the vibrissal afferent nerves, but not following vibrissae trimming. These results are dicussed in reference to the tonic or level-setting function of afferent systems.

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REFERENCES

1. ADAMETZ, J.H.: Rate of recovery of functioning in cats with rostral reticular lesions. *J. Neurosurg.*, **16**: 85-98; 1959.
2. BULLOCK, T.H. Tonic functions of afferents systems: An evaluation of implied cocepts. In: WENZEL, B.M. AND ZEIGLER, H.P. (Eds.) *Tonic Functions of Sensory Systems. Ann. N.Y. Acad. Sci.*, **290**: 35-42, 1977.
3. BULLOCK, T.H. AND HORRIDGE, A. *Structure and Function of the Nervous Systems of Invertebrates*. W.H. Freeman & Co., San Francisco and London. Vol. I, pp. 322-323, 1965.
4. COHEN, M.J. The dual role of sensory systems: Detection and setting central excitability. *Cold Spring Harbor Symposium on Quantitative Biology*, vol **30**: *Sensory Receptors*. Cold Spring Harbor, New York, pp. 587-599, 1965.
5. CROCKER, A.D. AND RUSSELL, R.W. The up-and-down method for the determination of nociceptive thresholds in rats. *Pharmacol. Biochem. Behav.*, **21**: 133-136, 1984.
6. DÉCIMA, E.E. AND PRCHAL, A. Effects of transitory blockade of vibrissae on rat behavior. *Society for Neuroscience, Abstr.*, **21**: 380, 1995.
7. DIXON, W.J. The up-and-down method for small samples. *Am. Stat. Assoc. J.*, **60**: 967-978, 1965.
8. GRANIT, R. *Receptors and Sensory Perception*. Chap. 3: Spontaneous Activity in Sense Organs and its Functional Significance. Yale University Press, New Haven, pp. 84-95, 1955.
9. FINOCHIETTO, E. AND FINOCHIETTO, R. *Técnica quirúrgica. Operaciones y aparatos*. Vol. II. *Anestesia local*. Edlar, Buenos Aires, pp. 35, 1944.
10. MELARAGNO, H.P. AND MONTAGNA, W. The tactile hair follicles in the mouse. *Anat. Res.*, **115**: 129-149, 1953.
11. MEYER, D.L. AND BULLOCK, T.H. The hypothesis of sense-organ-dependent tonus mechanisms: History of a concept. In: WENZEL, B.M. AND ZEIGLER, H.P. (Eds) *Tonic Functions of Sensory Systems*. Ann. *N.Y. Acad. Sci.*, **290**: 3-17, 1977.
12. PRCHAL, A., ALBARRACÍN, A.L. AND DÉCIMA, E.E. Blockage of vibrissae afferents: I Motor effects. *Arch. ital. Biol.*, **142**: 11-23,2004.
13. RICHARDSON, F. A study of sensory control in the rat. *Psychol. Rev. Monogr.* Suppl **12**: 60-100, 1909.

an increase in thresholds to footshock, after vibrissae afferent blockade, but not after trimming these tactile hairs. Therefore, it is likely that the loss in aggressive responses reported by Thor and Giselli (16, 17) was also due, at least partially, to increases in nociceptive thresholds to footshocks.

Lack of sensory information about the environment is the most obvious and simplest explanation for immobility and other related motor behavior changes present after afferent input suppression. On the other hand, any animal psychophysical study assesses sensory functioning by reference to behavioral (motor) responses. Thus, it could also be suggested that our results on footshock thresholds were related to the immobility produced by infraorbital nerve blockade. The question is whether the motionlessness observed in situations without specific stimuli (maze in Vincent's [19] case or open field in our case [12]) and the reaction to mild noxious stimuli (as is the case for the footshocks we used) are the same. The question is difficult and we are unable to answer it. In any case, however, because vibrissae trimming did not increase threshold values, we think that lack of tactual information present in the situation of vibrissae block cannot be used to explain our data.

It is extremely difficult to fit these results into current neurophysiological theoretical schemes. It is very hard to explain how local anaesthesia of the face can produce a severe depression of flexor reflexes evoked by stimuli applied to the feet. As far as we know, there are only two papers whose data are similar to ours. Von Uexküll (18) reported a slow loss of muscle tone and responsivenees to mechanical stimuli in *Sipunculus nudus* when the animal deprives itself of possible sensory inputs by staying in its burrow. After removing the animal from the burrow, responsiveness to sensory stimulation and muscle tone are slowly regained. In the same line, Cohen (4) reported that the crab (*Cancer magister*) is less responsive to external stimuli such as food or moving objects after the removal of several myochordotonal organs (a proprioceptive receptor of the walking legs of this crab).

The footshock threshold rises produced by IO blockage could not be due to a specific effect of these treatments, i.e., the lack of trigeminal sensory information could not explain the threshold increments to foot stimulation. The possibility that procaine injected in vibrissal pads would affect other afferent systems or the CNS (diffusing perhaps through bone holes) is remote and was contested. The effects of mechanical blockage of vibrissae (by forced injection of saline in the pad) is a very strong argument against this type of criticism. Instead our results may be interpreted as a generalized or non specificic depressive effect of vibrissal afferent blockage. In this way we think that the "tonic hypothesis" is the simplest and the most comprehensive explanation for our data.

As in the motor behavior case (12), the differences in threshold increments between permanent and transitory blockade groups could be explained by the well known capacity of the central nervous system to recover after time from different kinds of acute lesions. In this regard our results again agree with Thor and Giselli (17), who reported that there was a recovery to normal levels of fighting when vibrissae were anaesthetized repeatedly (daily). The rat's capacity to overcome vib-

injection of physiological solution into the pads did not produce any obvious signs of pain (e.g. vocalization).

Besides the loss of both vibrissal movements and pad sensibility, this mechanical blockage produced the same general effects as the procaine injection: general hypomotility (even immobility), slowing of all movements and an apparent loss of muscular tone. The effects were also perfectly reversible and the duration of the mechanical anaesthesia, as expected, was shorter than the chemical one.

b) *Footshock thresholds*

The flinch response values obtained in this experiment for normal rats (Mean = 0.15 mAmp, standard deviation = 0.02) were similar to the values reported by

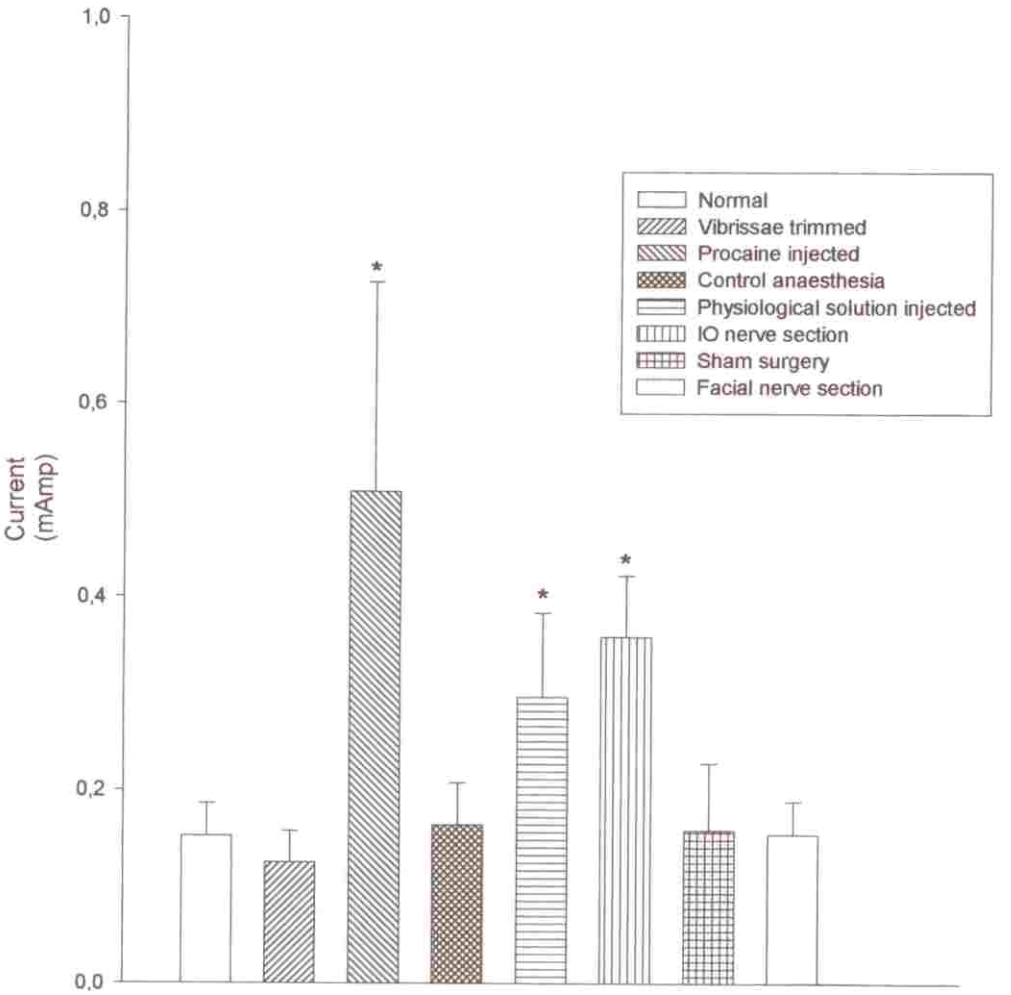

Fig. 1. - *Threshold values for flinch responses.*
* $p < 0.01$

METHODS

Animals

Male and female adult Wistar rats (250-300 gr) were used. Rats were housed in a experimental room with 12 hs light-dark cycle, (light on at 7.00 AM) for a week prior to experimental manipulation and threshold tests. Food and water were available ad libitum.

Eight experimental groups were tested:

1) Normal group (N = 14): rats without any treatment.
2) Vibrissae trimming (N = 8): all vibrissae on both sides were trimmed at the level of the fur, just before the start of the sesion.
3) Transitory blockage of vibrissal afferents (N = 10): this was produced by local injection of Procaine (0.5% in saline). One cc was injected in each vibrissal pad. Rats were tested 2 min after the injection.
4) Control anaesthesia (N = 8): to assess the possible role of systemic absorption of procaine, similar amounts of the drug (previous group) were injected subcutaneously in the rat dorsum. The test was also carried out two min. later.
5) Mechanical blockage of vibrissal nerves (N = 7). There is the remote possibility that procaine injected in the vibrissal pads could also affect other close afferent systems (as the olfactory and/or the ocular). On the other hand, the presence of the vibrissal follicles create a situation potentially different than that present in the previous group (procaine injected in the dorsum). The existence of large blood sinuses typical of the vibrissal follicles could result in larger levels of systemically absorbed procaine in the former than in the control group. In order to obviate these factors, a mechanical system, for blocking vibrissal afferents by compression was used in this group. Thus, 1.5-2 cc of physiological solution was injected in each side on the tissue pad. Effectiveness of this mechanical nerve block was behaviorally assesed (see results). Footshock thresholds were tested two min after injection.
6) Permanent blockage (N = 11): both IO nerves were cut under general anaesthesia (Pentobarbital 40 mg/kg, i.p.). Prior to the neurotomy 0.3 cc of Procaine solution (1%) was injected inside the nerve trunk. The IO nerve was severed just when it emerges from the infraorbital fissure. In this case, rats were tested 22-26 hs after the operation.
7) Facial nerve section (N = 8) Local anaesthesia blocks both afferent and efferent vibrissal innervation. Furthermore, vibrissal pad movements remain unchanged after cutting the whiskers. Thus, to evaluate the importance of motor activity, the facial nerve branches supplyng vibrissae muscles were severed. under general anaesthesia (Pentobarbital 40 mg/kg, i.p.). Section of both buccal and upper divisions of the marginal mandibular branches of the facial nerve, were made 1-2 mm rostral to the extraorbital lacrimal gland. Rats were also tested 22-26 hs later.
8) Sham group (N = 10): same procedures as in groups 6 and 7, but without nerve section. Sham surgery for group 6 (IO nerve section) included troncular anaesthesia. Rats were also tested 22-26 hs after the operation.

Rats in IO and facial nerve sectioned groups were killed by decapitation, under ether general anaesthesia after the experiments were carried out in order to check for completness of nerve section. Animals with intact nerve fibers were not considered in the final analysis of the results.

Apparatus and procedures.

These experiment were based on the design used by Crocker and Russell (5) for testing nociceptive thresholds. According with their instructions, a test chamber made of transparent acrylic (30x30x30 cm) was used. The floor consisted of stainless steel bars (0.28 cm diameter; spaced 1.2 cm apart). Shocks were delivered by a pulse generator (also constructed in our Lab.) connected to the floor's bars. Available intensities ranged from 0.018 to 3.72 mA, arranged in a 24-step-logarithmic scale. The full range was never used in determining thresholds. Each pulse had a duration of 0,5 sec., pulses were delivered at 10 sec. intervals, and their intensity was controlled by the experimenter. In all cases two trained observers, situated in front of two adjacent sides of the test chamber, decided occurrence or non-ocurrence of footshock responses. The floor of the test chamber was placed at eye level so that a full view of the animal's paws was assured.