Brain Research, 332 (1985) 247–257
Elsevier

BRE 10676

Comparison of Deficits in Electrical Self-Stimulation After Ibotenic Acid Lesion of the Lateral Hypothalamus and the Medial Prefrontal Cortex

S NASSIF, B CARDO, F LIBERSAT and L VELLEY

Laboratoire de Psychophysiologie, Université de Bordeaux I, 33405 Talence Cedex (France)

(Accepted July 23rd, 1984)

Key words self-stimulation — lateral hypothalamus — medial prefrontal cortex — intrinsic neurons — ibotenic acid

The aim of the present study was to compare the self-stimulation deficit produced by a unilateral injection of the neurotoxin, ibotenic acid, in the lateral hypothalamus (LH) to the deficit produced by the same unilateral injection in the medial prefrontal cortex (MPC) Four groups of adult male Sprague–Dawley rats were used in two control groups, electrodes were bilaterally implanted in the LH (5 rats) or in the MPC (6 rats) and self-stimulation (ICSS) was obtained separately with the right and left electrodes In the two experimental groups the intrinsic neurons of the LH (8 rats) or of the MPC (10 rats) were destroyed unilaterally by local injection of ibotenic acid (4 μg in 0 5 μl), the other side served as the sham-lesioned control Ten days later ICSS electrodes were implanted bilaterally, one in the lesioned area, the other in the contralateral region As in the case of the control rats, ICSS was determined separately for each electrode, first by a rate dependent test (nose-poke) then by a 'rate-free' test (shuttle-box) In the LH and MPC control rats, ICSS responses were the same with stimulation on either side In the LH-lesioned rats, the ICSS rates measured with the nose-poke test were significantly decreased with stimulation on the lesioned side, whereas rates with stimulation of the non-lesioned LH were normal Likewise, while shuttle responses with stimulation of the non-lesioned LH were normal, the OFF-time was increased and the ON-time was decreased with stimulation of the lesioned LH In the MPC-lesioned rats, ICSS (nose-poke) was totally suppressed and the shuttle responses were disorganized since neither the ON- nor the OFF-times changed in response to increasing current intensities Nose-poke responses with stimulation of the non-lesioned MPC were just about normal These results show that in the two brain regions studied local neurons are involved in ICSS The difference in the magnitude of the deficit observed suggests, that the neuronal circuits involved in MPC self-stimulation are poorly represented whereas in the LH many neuronal circuits involved in these mechanisms overlap

INTRODUCTION

In a previous paper, we showed that the unilateral lesion of the intrinsic neurons located in the lateral hypothalamus (LH) of the rat, by local injection of 4 μg of the neurotoxin, ibotenic acid (IBO) produced a long-lasting suppression in intracranial self-stimulation (ICSS) with electrodes in the lesioned region without modification of ICSS in the contralateral LH[56] Biochemical assays showed that at the dose used the lesion did not produce any decrease in the levels of dopamine, noradrenaline and serotonin in regions of the brain rostral to the lesion Taken together, these results indicated that the intrinsic neurons of the LH are involved in ICSS and that, in the absence of these neurons, the main aminergic fibers do not seem to be able to support ICSS

In doing the present experiments, we wanted to answer two main questions raised by these results The first question concerns the reward–performance distinction In our experiments, ICSS was tested by using a nose-poke response However, since Valenstein's first studies (see review in ref 53), it has become generally accepted that the traditional lever-pressing method confounds reward and performance effects (see review in ref 30) since the rate of self-stimulation can be modified by various factors other than reward Now, nose-poking is as much a rate-dependent measure as is lever-pressing Consequently, we cannot be certain, for the moment, that the impairment in ICSS which we observed after unilateral lesion of the LH is due to a reward deficit. In 1962,

Correspondence L Velley, Laboratoire de Psychophysiologie, Université de Bordeaux I, Avenue des Facultés, 33405 Talence, Cedex, France

Olds[39] suggested that the intrinsic neurons of the LH were the main substrate of reward, but in 1977[40] he proposed that these cells were 'drive' neurons. In fact, the role of this neuronal population in LH self-stimulation is still not known.

Although the casual observation of the unilaterally lesioned rats did not show any gross behavioral impairment, the possibility of subtle performance deficits could not be excluded. In order to investigate more closely the kind of deficit which might be produced by unilateral IBO lesion in the LH, we decided to compare the effects of this lesion on two different ICSS measures, nose-poke behavior and a 'rate-free' task first proposed by Valenstein and Meyers[54] (see review in ref. 30). In this latter situation, rats are taught to turn the brain stimulation ON and OFF by moving back and forth in a 'shuttle-box'. Various arguments suggest that the processes determining the two measures normally taken in the shuttle-box — the latency to initiate the stimulation (OFF-time) and the latency to turn off the stimulation (ON-time) — are independent and that the OFF-time reflects reward processes[2,4,9,12,18,31,32,47]. The meaning of the ON-time is more controversial since, for some authors, this duration indicates an increase in an aversive effect of the stimulation[3,5,6,24,36,47], while for others, the ON-response reflects adaptation of the reward effect[15,16]. Whatever the precise meaning of shuttle-box responses, we assumed that if unilateral IBO lesion reduced the reward value of the stimulation, the OFF- and ON-times would be differentially affected. On the other hand, if the lesion produced a non-specific deficit, the two measures would be modified in the same way.

The second question raised by our previous work relates to the role of local neuronal circuits in ICSS. Our previous results are in agreement with various other observations suggesting that LH ICSS is mediated, at least in part, by local neuronal populations and not by some wide-spread, unitary reward system[26,50–52]. If this hypothesis is right, ICSS in other brain regions will also be dependent on the intrinsic neuronal circuits located in the stimulated area. Since the first observations by Routtenberg[42,43] it is well-known from mapping studies that ICSS can be obtained in two regions of the rat prefrontal cortex: the medial prefrontal area and the sulcal or suprarhinal region. This, together with the observation that the dopaminergic fibers coming mainly from the ventral tegmental area project to these two regions[1,7], has led various authors to propose that these dopaminergic projections mediate the reinforcing properties of ICSS[13] (see review in ref. 57). However, this hypothesis was not confirmed[20,29,48] and some recent studies suggest that prefrontal ICSS is mediated by neuronal populations located in the prefrontal cortex proper (cf. Discussion). Consequently, we decided to investigate the later hypothesis by comparing ICSS of each of the two medial prefrontal cortices (MPC) of the same rats after IBO lesion of the intrinsic neurons of one side only. As in the case of the LH-lesioned rats, ICSS of the MPC-lesioned animals was tested using both the nose-poking and the shuttle-box methods. This experiment also allowed us to compare the local IBO lesion effects on ICSS of MPC with the effects on ICSS of the LH.

MATERIALS AND METHODS

Male rats of Sprague–Dawley strain were housed in individual cages in a temperature-regulated (23 °C) animal room on a normal 12 h–12 h light/dark cycle. They were given ad libitum access to food and water. Surgery was performed when the rats were 3 months old.

Lesioned rats

A two-stage operation was performed. Under Nembutal anesthesia (40 mg/kg i.p.) two cannulae, 0.2 mm in diameter, were implanted in the LH for 10 rats and in the MPC for 10 rats under stereotaxic control. The coordinates were the following: LH: 1.8 mm posterior to Bregma, ± 1.6 mm lateral to the sagittal suture and 8.8 mm ventral to the top of the skull, MPC: 3.7 mm anterior to Bregma, ± 0.7 mm lateral to the sagittal suture and 3.7 mm ventral to the top of the skull. The incisor bar was level with the interaural line.

The cannulae were connected to a micro-pump which always delivered IBO to one cannula and vehicle solution simultaneously to the other cannula. Half of the injections of IBO were made on the right side and half on the left. All rats were injected with 4 μg of IBO in 0.5 μl of vehicle (phosphate buffer). The injections were made over 6 min; 30 s and 10 more min elapsed before removal of the cannulae. Between 10

and 20 days after the injection, the rats again underwent surgery and two bipolar electrodes were implanted bilaterally using the same coordinates as for the cannulae. Each electrode consisted of two twisted, insulated platinum-iridium wires 0.09 mm in diameter.

Control rats

Fourteen rats of the same age were treated exactly as the IBO injected animals (LH: 7; MPC: 7) but during the first operation the two sides received the vehicle only (0.5 μl).

Behavior

ICSS was tested first by the nose-poking method, then in the shuttle-box.

Nose-poking behavior[55]. A cylindrical metal container, 25 cm in diameter was used. Two holes, 1.5 cm diameter, were located 10 cm apart on the wall, 5 cm above the floor. Outside the apparatus, two small infrared photocells were located in front of the holes in such a way that each nose-poke interrupted the beam. During ICSS trials, one of the photocells was connected to a stimulator and each nose-poke into this 'active' hole resulted in the delivery of brain stimulation (0.2 s sinusoidal current, 100 Hz). The other hole (inactive hole) was not connected to the stimulator, but nose-pokes were also automatically counted. The electrodes were connected to the stimulator by way of a mercury commutator.

The testing began at least 10 days after the implantation. Electronic switching permitted separate stimulation of either electrode. The two electrodes were thus always tested separately.

For half of the control rats, testing began on the right, for the other half, on the left. For the IBO-lesioned rats, testing began with the electrode on the non-lesioned side. For the first 3 days, each rat, both control and lesioned, was trained to nose-poke for 20 μA stimulation (peak to peak) during 15-min sessions until rates stabilized. If responses could not be obtained at 20 μA (which was the case for the lesioned side only) the intensity was increased by 10 μA steps up to 60 μA. After this preliminary screening, ICSS was measured for each electrode at the following intensities: 10, 20, 30, 40, 50 and 60 μA. For each intensity, performance was measured for 5 min from the first spontaneous nose-poke. Then, the active hole became inactive and vice-versa and performance was again measured for 5 min. At least two different tests were given for each electrode. The intensities were applied in an ascending order. An electronic counter recorded, at 5-min intervals, the number of stimulations received as well as the number of the nose-pokes into both the active and inactive holes.

Shuttle-box behavior. Animals were tested in a clear Plexiglas box (50 × 21 × 35 cm) with a grid floor. At each end of the box, photocells were positioned 7.5 cm from the end wall and 3.7 cm above the grid floor. A micro-computer controlled the experimental conditions so that interruption of one photocell beam caused sinusoidal current (100 Hz) to be delivered continuously through one bipolar electrode until the photocell beam at the other end of the box was interrupted by the rat. Each session lasted 10 min. The computer recorded each latency to turn on the stimulation (OFF-time) and each latency to turn it off (duration of stimulation or ON-time). At the end of each session, individual OFF- and ON-times, the means of these times, the number of crossings and the total OFF- and ON-times were printed out.

Initial training began two days after the end of the nose-poking experiments. Each electrode of each rat was tested at 30 μA for 10 min until performances stabilized. After this preliminary screening, shuttle-box responses were measured for each electrode at various intensities (15, 20, 25, 30, 40 and 60 μA). For the LH rats only, two additional current intensities (80 and 100 μA) were also tested. These measures were repeated at least twice. At the end of the experiment, the rats were killed and the position of the electrode-tips and the extent of the lesion were determined from 80 μm frontal sections of the brain stained with thionine. Analysis of variance with repeated measures was used to analyze the data. A number of rats, controls or lesioned, were discarded at the end of the experiment, after the histological control. Two LH control rats did not self-stimulate with one of the two electrodes, for one of these rats the electrode was short-circuited, for the other, one of the electrode tips was in the internal capsule. Two rats lesioned in the LH did not self-stimulate with either of the two electrodes. The histological analysis showed that the electrodes of these rats were outside the brain. In the case of the MPC, one control rat was eliminated due to a short circuit in one electrode.

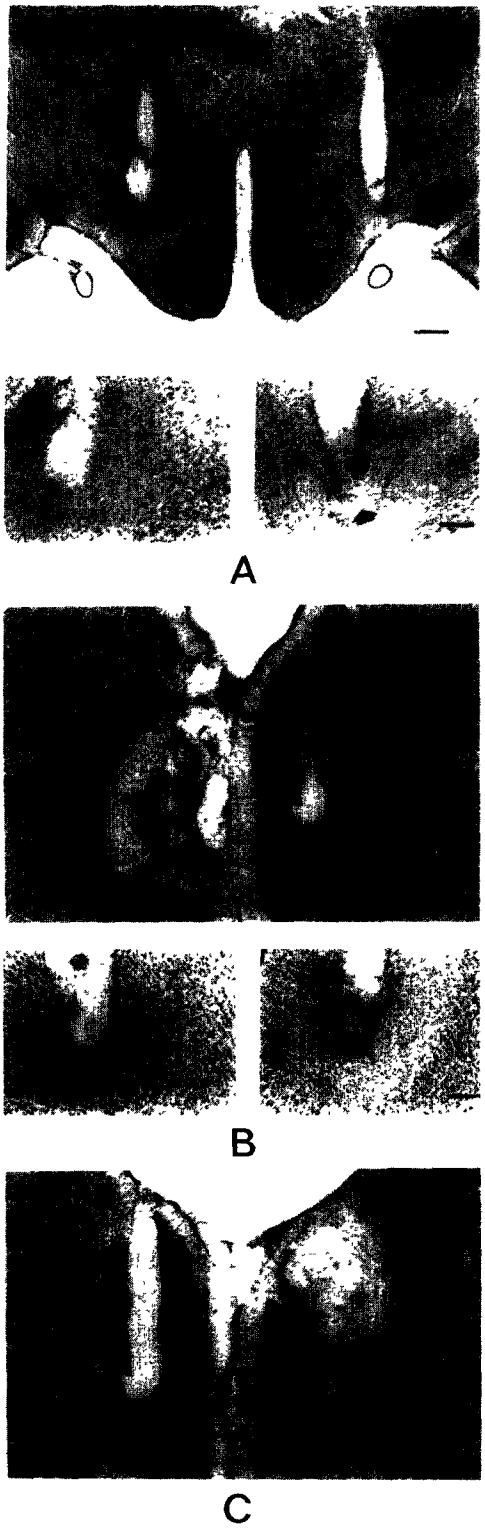

Fig 1 Examples of ibotenic acid lesion A top, rat 1741 lesion in the right LH, bottom, rat 1739 lesion in the right LH, enlargement of the region around each electrode tip B top, rat 1699 lesion in the left MPC, bottom, rat 1674 lesion in the right MPC, enlargement of the region around each electrode tip C rat 1705 for this rat the ICSS performance was the same for the two sides The electrode tip in the lesioned MPC (right side) was below the lesioned area Scale-bars 0 5 mm for the bilateral views, 0 2 mm for the other figures

RESULTS

Histological analysis

In the LH, all electrode tips were located between the fornix and the internal capsule The variability of the implantation sites in the antero-posterior plane was 0 9 mm. The most anterior electrodes were located in plane 4890 of the atlas of Konig and Klippel[27] and the most posterior electrodes were found in plane 3990 A complete loss of neurons associated with an extensive glial proliferation, was observed in the lesioned area In the medio-lateral plane, the lesion extended from the perifornical region to the internal capsule. The medial hypothalamic nuclei were not lesioned. In the dorso-ventral plane, the lesion extended from the zona incerta to the bottom of the brain in 4 brains only For the 4 other brains, viable cells were observed in the ventral part of the LH. The zona incerta was always damaged. In the antero-posterior plane, the extent of the lesion varied between 1400 μ and 1900 μ. For 6 brains the lesion began in plane 5150 of the Konig and Klippel atlas, the two others began in planes 5340 and 5660 Posteriorly, 5 lesions ended in plane 3430, two in plane 3750 and one in plane 3990 In all cases the stimulation electrode was located in the lesioned area In 4 brains the ventral thalamus was also damaged We observed neither cell-loss nor lesion in areas distal to the site of the lesion, with special attention given to the hippocampus

In the MPC all electrode tips but one were located in the two planes 10300 and 10500 of the Konig and Klippel atlas Both of the electrodes of one rat were located in plane 11050 The MPC lesions did not have the shape as those of the LH The lesions were elongated in the dorso-ventral plane, the height varied between 1 and 3 mm and in the medio-lateral plane the extent of the lesion did not exceed 1 mm. In the antero-posterior plane the extent varied between 720 and 1840 μ. Following the Krettek and Price classification[28] in every case, the lesion destroyed the dorsal division of the anterior cingulate area and the prelim-

bic area For 8 of the 9 brains the electrode was located in the lesioned area, but for one brain the electrode had traversed the lesioned region and the tip came to rest in the non-lesioned cortex (Fig 1C) As it happens, the ICSS behavior of this rat with stimulation through this electrode was normal, unlike all the other lesioned rats (see below) Consequently, the ICSS data for this rat were included with the data of the MPC control rats.

Behavioral results

The unilateral lesion in the LH as well as in the MPC did not produce any visible deterioration and food intake was normal 24 h after the operation All LH rats, lesioned and control completed both tests However, during the shuttle-box test, 4 MPC rats (3 control and 1 lesioned) were discarded: for two rats the electrodes were dislodged and for the two others one of the electrodes became short-circuited

The LH control rats as well as the LH-lesioned rats (non-lesioned side) learned the two tests quickly and performance was stabilized after 3–5 sessions. The MPC rats, on the other hand, learned ICSS very slowly With the nose-poke test, most of the rats began to self-stimulate after 3–4 sessions, but for some rats significant ICSS (100 responses in 10 min) appeared only after 10 sessions. For the control rats and for the non-lesioned side of the lesioned rats, 10 to 12 more sessions were necessary in order to stabilize performance (300–400 responses in 10 min). Likewise in the shuttle-box, 7–12 sessions were necessary before the performance of MPC rats stabilized

Moreover, in the MPC control rats it was noted that the acquisition of the nose-poking behavior was very rapid when the stimulation switched to electrode tested second (compared to acquisition with the electrode tested first during the initial stabilization), that is, the transfer of ICSS from one hemisphere to the other appeared to be immediate

Nose-poking behavior Fig 2 summarizes the results obtained with this test and allows us to compare the effects of unilateral LH lesion (left column) with the effect of a unilateral MPC lesion (right column) on ICSS The data obtained with the control rats are in agreement with other published results For the LH rats, the increase in intensity produced a large increase in the response rate and the performance observed after stimulation of each LH of the same rat

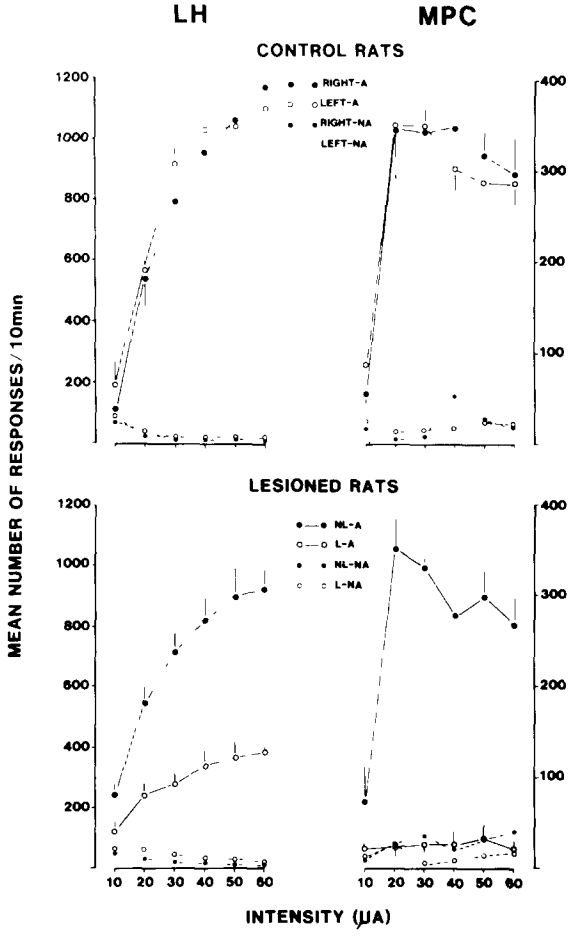

Fig 2 ICSS measured by the nose-poke response left, LH ICSS, right, MPC ICSS Top, control rats (LH n = 5, MPC n = 7), A, active hole, NA, non-active hole Bottom, lesioned rats (LH n = 8, MPC n = 9), NL, non-lesioned side, L, lesioned side Since the active hole was switched after 5 min, for each rat, the numbers of nose-pokes made at each hole were summed over a 10-min period For clarity, the S E M s of the mean values of nose-pokes at the non-active hole are not shown Note the different scales for LH rats (left ordinate) and for MPC rats (right ordinate)

was not different since the two curves do not differ significantly ($F_{1\,8} = 0\ 14$, n.s) Likewise for the control MPC rats there was no significant difference in the rate of nose-poking between the two hemispheres ($F_{1\,12} = 0\ 06$, n s) As noted by others[21 41] an increase in intensity did not produce an increase of response-rates, since the best performance was observed at 20 μA

The curves presented at the bottom of Fig 2 show the effects of IBO lesions. For the LH-lesioned rats, the nose-poking observed with stimulation of the non-lesioned side did not significantly differ from the

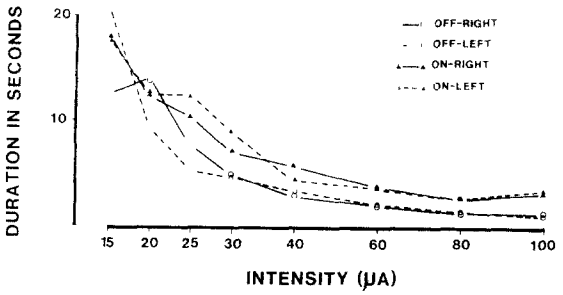

Fig 3 Mean ON- and OFF-durations with stimulation of the right and left lateral hypothalami of the control rats (n = 5) For clarity, S E M values are not shown on this and the following figures

scores recorded with stimulation of either LH of the control rats ($F_{2,15} = 0\ 8$, n.s) On the other hand, ICSS of the lesioned side was significantly lower than ICSS of the non-lesioned side ($F_{1\ 14} = 32.8$, $P < 0\ 001$). Likewise, for the MPC-lesioned rats, the ICSS rates obtained with stimulation of the non-lesioned side were not significantly different from those recorded with stimulation of either hemisphere of control rats ($F_{2,20} = 0\ 14$, n.s) However, ICSS of the lesioned side was totally abolished since the scores not only differed significantly from those observed with stimulation of the non-lesioned side ($F_{1\ 16} = 84\ 07$, $P < 0\ 001$), but also did not differ significantly from the responses made to the non-active hole ($F_{1\ 16} = 1\ 3$, n s)

Shuttle-box behavior. Fig. 3 shows the mean durations of the ON- and OFF-responses measured with stimulation of each LH of the control rats Neither the difference between the two ON-times, left and right, nor the difference between the two OFF-times reached significance (ON-time, $F_{1\ 8} = 1\ 03$, n s OFF-time $F_{1\ 8} = 0\ 01$, n s)

Fig. 4 allows us to compare the shuttle performance observed with stimulation of each LH in the 8 lesioned rats The ON and OFF-times for the non-lesioned LH (left) do not differ from those obtained with stimulation of either side of the control rats (Fig 3) (ON-time $F_{2\ 15} = 0\ 2$, n.s . OFF-time· $F_{2\ 15} = 0\ 01$, n.s) For the lesioned side (right) the mean number of crossings does not differ from that observed for the non-lesioned side ($F_{1\ 14} = 0\ 56$, n.s), but the mean OFF-time was significantly increased compared to the OFF-time of the non-lesioned side ($F_{1,14} = 7.9$, $P < 0\ 02$) Conversely, the ON-time is decreased ($F_{1\ 14} = 7\ 3$, $P < 0\ 02$)

Fig. 5 shows the mean ON- and OFF-times observed with stimulation of each MPC in control rats Neither the ON-times nor the OFF-times differ between the two sides (ON-time· $F_{1\ 6} = 0\ 04$, n.s., OFF-time $F_{1,6} = 0\ 55$, n.s)

Fig. 6 compares the mean ON- and OFF-time obtained with stimulation of each MPC in lesioned rats For the non-lesioned side, the OFF-time does not differ from the OFF-time observed with stimulation of

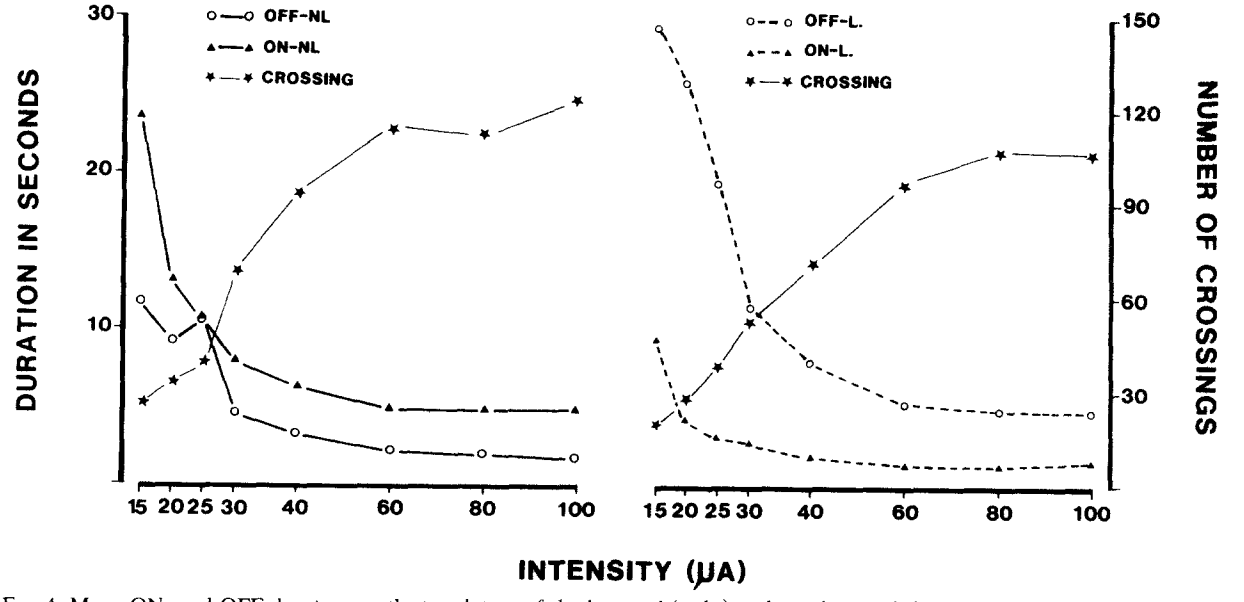

Fig 4 Mean ON- and OFF-durations with stimulation of the lesioned (right) and non-lesioned (left) lateral hypothalami of the lesioned rats and mean number of crossings (n = 8)

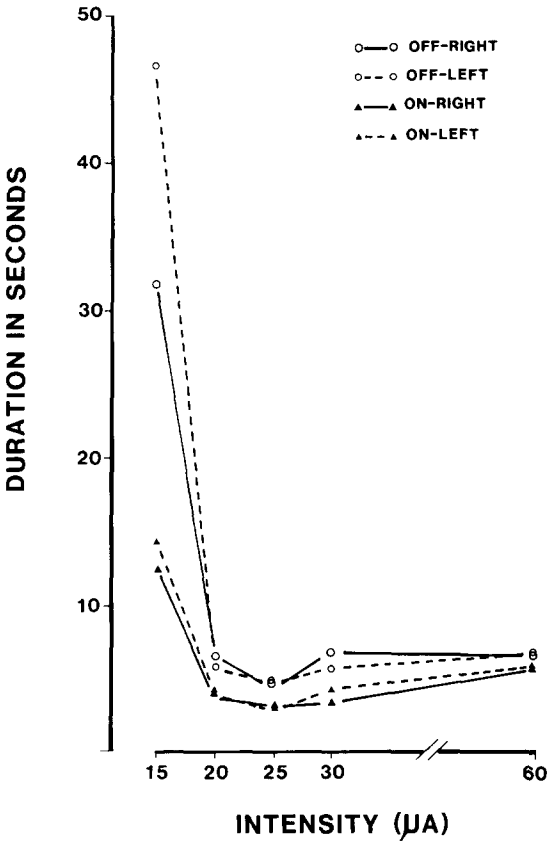

Fig 5 Mean ON- and OFF-durations with stimulation of the right and left medial prefrontal cortices of the control rats (n = 4)

either MPC of the control rats ($F_{2,12} = 0.64$, n s.). However, the same comparison for the ON-time shows that this variable measured with stimulation of the non-lesioned MPC is significantly increased ($F_{2,12} = 8\ 9$, $P < 0.01$) As for the responses with stimulation of the lesioned side, they were disorganized the ON-time did not decrease with increasing current intensities Likewise, the OFF-time was variable and did not show systematic modification. For the ON-time the comparison between the two sides is significant ($F_{1\ 12} = 9\ 05$, $P = 0\ 01$) but because of the great variability in OFF-times, the comparison between the two sides does not quite reach significance ($F_{1,12} = 4\ 04$, $P = 0\ 065$) The number of crossings, with stimulation of the lesioned side, was very small and varied between 14 at 15 μA and 8 at 60 μA.

DISCUSSION

The aim of the present experiments was to compare ICSS of the two LH or of the two MPC of the same rat after IBO lesion of the intrinsic neurons of one side (LH or MPC) only. Moreover, rats bilaterally implanted in the symmetrical area (LH or MPC) but not lesioned served as non-lesioned controls In order to better understand the kind of deficit produced by the lesion, two different ICSS tests were used: a rate-dependent test and a rate-free test.

Using the nose-poke test the results obtained after a unilateral lesion of the LH agree with our previous data[56] Whereas ICSS in the non-lesioned LH is normal, the behavior observed with stimulation of the lesioned LH is significantly decreased The fact that ICSS of the unlesioned side is not modified shows that the deficit observed with stimulation of the lesioned LH is not due to some general debility produced by the lesion The results obtained with the shuttle-box test are in agreement with the nose-poke data· the ON- and OFF-times recorded with stimulation of the unlesioned LH are the same as the ON-

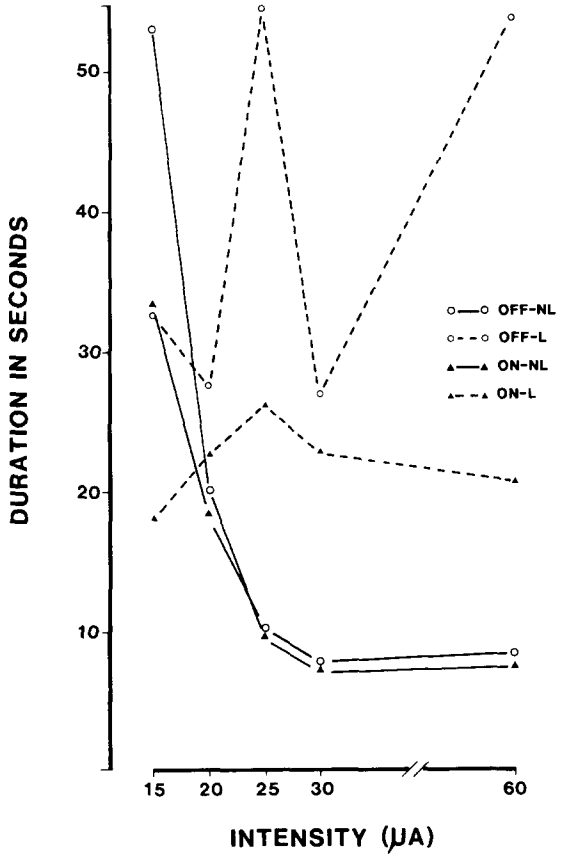

Fig 6 Mean ON- and OFF-durations with stimulation of the lesioned and non-lesioned medial prefrontal cortices of the lesioned rats (n = 8)

and OFF-times obtained with stimulation of each LH in the control rats. However, for the lesioned LH the OFF-time is significantly increased and conversely the ON-time is decreased. Since most authors agree that the OFF-time reflects reward processes (see introduction) we may assume that it is these processes which are depressed by the IBO lesion. The simultaneous decrease in ON-time is more difficult to explain since the meaning of this response is controversial (see introduction). However, in our experimental conditions, the ON- and OFF-responses were modified in opposite ways. Furthermore, the number of crossings obtained with stimulation of the lesioned LH was the same as that recorded for the non-lesioned LH. Thus, since the motor organization of the behavior was normal and since the ON- and OFF-times were differentially affected, we can assume with some confidence that reward processes are specifically affected by the lesion.

However, the present results, just like our previous data[56] show clearly that self-stimulation is only depressed by a unilateral lesion and not abolished, in spite of the fact that all local neurons around the stimulation electrode are lost. Consequently, what is the substrate for the remaining ICSS of the lesioned area? At present the neuronal substrate of LH self-stimulation is not known. The LH is a heterogeneous region comprising, besides the intrinsic cells, all the ascending and descending fibers of the medial forebrain bundle (MFB)[34,37,38]. Some recent electrophysiological data obtained using the 'collision test' have shown that collision effects can be observed when the two electrodes are located along the MFB. These results are interpreted as showing that electrodes anywhere in the MFB tap into a common substrate[10,19].

These findings are consistent with the results of a recent experiment indicating that 45 min of LH self-stimulation produces an increased uptake of [^{14}C]2-deoxyglucose along the whole MFB[58]. However, these results are not easy to reconcile with the data of Huston[26] indicating that LH self-stimulation is not changed after unilateral removal of all telencephalic structures and also after disconnection from the brainstem. Although these later findings are presently not easy to explain, they show clearly that the long ascending or descending fibers passing through the LH but originating outside this structure are not the main substrate of ICSS and that at least a part of the reward process is initiated in the LH proper. Taking into account all these findings, we propose the following explanation of our results. Our histological analysis shows that not all intrinsic neurons located in the anterior LH and in the posterior LH are destroyed. Moreover, it is well-known from tritiated amino-acid studies and from horseradish peroxidase studies that all parts of the LH contribute ascending and descending fibers to the MFB[8,25,44]. Thus at present it seems that most of the cells located in the LH have long axons, some of them projecting outside the hypothalamus. Consequently, in our experimental conditions, the axons ascending from the posterior LH cells as well as the axons descending from the anterior LH cells can be stimulated by the electrode since they pass through the lesioned area. In other words, we assume that residual ICSS observed in the lesioned area is supported by fibers coming from the viable intrinsic cells located in front of or caudal to the lesion. Experiments are currently in progress in order to test this hypothesis.

Compared with the effect observed in the LH, the deficit in ICSS of the MPC is more dramatic: no self-stimulation could be obtained with the nose-poke test, and the shuttle response was disorganized, since neither the ON-time nor the OFF-time showed systematic variation with increasing current intensities. However, as in the case of the LH, ICSS of the unlesioned MPC is normal, although the ON-time is significantly increased when compared with the ON-time measured in a control rat. However, this isolated finding requires confirmation. For the moment we can conclude that the major ICSS deficit observed with electrodes in the lesioned side cannot be explained by a general debilitation of the animals. Furthermore, if the electrode is located outside the lesion, ICSS is normal (Fig. 1C). This observation suggests that the deficit is due to the loss of the neurons located in the vicinity of the electrode tip. The role of prefrontal cortex neurons in self-stimulation has been studied by various authors using different methods. Gerfen and Clavier[20] showed that ICSS in the sulcal cortex is suppressed by local kainic acid injection. For these authors, this result supports the hypothesis of a descending corticofugal system originating in the sulcal cortex and supporting sulcal ICSS. Furthermore, electrolytic lesion of major input and output connections of the MPC (i.e. lateral hypothal-

amus, dorsomedial nucleus of the thalamus and nucleus accumbens) does not affect MPC self-stimulation, whereas bilateral parasagittal cuts between the MPC and the sulcal cortex eliminate ICSS from the MPC[14]

The independence of LH and MPC self-stimulation is confirmed by electrophysiological data showing that the neuronal refractory periods in the two regions are different and that the rewarding effects of stimulation do not summate when stimulating pulses are concurrently delivered to these two sites[45]. Likewise, in work already cited[58], it was shown by the [^{14}C]2-deoxyglucose method that the electrodes implanted in the LH and those implanted in the MPC do not activate the same neuronal pathway and that stimulation sites in the MPC produce increased radioactivity uptake only in the MPC proper, in the claustrum and in the basolateral nucleus of the amygdala Taken together, all these findings obtained by various methods are in agreement with our data and suggest that prefrontal ICSS is mediated by neuronal populations located in the prefrontal cortex and not by fibers passing through or projecting to this area

In summary our results show that local neurons in both the LH and the MPC are involved in ICSS, but the large difference in the size of the deficits observed can probably be explained by the fact that in the MPC the neuronal population mediating reward processes is smaller represented, whereas in the LH many neuronal entities involved in reward processes overlap and can be stimulated simultaneously

Lastly, we would like to introduce a cautionary note the interpretation of our results rests on the assumption that ibotenic acid destroys only cell bodies and not fibers of passage. However, the possibility that this neurotoxin produces a total lesion cannot be excluded, at least for certain doses injected. A recent finding[49] showed that LH self-stimulation is not modified by the injection of 1 μg of IBO dissolved in 1 μl At present, this discrepancy can be explained by the different dose of neurotoxin used since we injected 4 μg in 0 5 μl, i.e. a concentration 8 times greater than that used by Sprick et al [49] Thus, the possibility that the deficit observed in our conditions may be due to an undetected lesion of a few fibers cannot be excluded For example, it was reported that noradrenergic fibers are damaged by the neurotoxin, kainic acid[35] This observation was not confirmed[11] but it seems that kainic acid does produce demyelinization in the mesencephalic tegmentum[17] However, a similar effect has not yet been found for ibotenic acid Hansen et al [23] injected this neurotoxin in the preoptic area and in the LH at a 10 μg/μl concentration and did not observe any lesion of the aminergic fibers Moreover, the injection of large doses of IBO, up to 20 μg in 1 μl, into the striatum did not damage aminergic fibers and myelinated bundles[22,46] Our previous results[56] similarly confirmed that the injection of 4 μg or 6 μg of IBO into the LH does not produce significant loss of amines in the striatum and in the hippocampus In conclusion, it is possible that future findings will show an action of this neurotoxin on some fibers, but the presently available data do not indicate any lesion of fibers of passage after IBO injection.

ACKNOWLEDGEMENTS

This investigation was supported by C N R S. (L A no 339). We thank Dr Caudarella for correcting the English text and M. T Roy, J Ducout, C Darracq, A. M Perret and M Saint-Marc for technical assistance

REFERENCES

1 Albanese, A and Bentivoglio, M , The organization of dopaminergic and non-dopaminergic mesencephalocortical neurons in the rat, *Brain Research*, 238 (1982) 421–425
2 Atrens, D M , Reinforcing and emotional consequences of electrical self-stimulation of the subcortical limbic-forebrain, *Physiol Behav* , 5 (1970) 1461–1471
3 Atrens, D M and Becker, F T , Assessing the aversiveness of intracranial stimulation, *Psychopharmacologia*, 44 (1975) 159–163
4 Atrens, D M and Sinden, J D , Anatomically-dependent facilitation and inhibition of hypothalamic self-stimulation by food deprivation, *Behav Biol* , 13 (1975) 225–231
5 Atrens, D M , Becker, F T and Hunt, G E , Apomorphine selective inhibition of the aversive component of lateral hypothalamic self-stimulation, *Psychopharmacology*, 71 (1980) 97–99
6 Atrens, D M , Sinden, J D and Hunt, G E , Dissociating the determinants of self-stimulation, *Physiol Behav* , 31 (1983) 787–799
7 Berger, B , Thierry, A M , Tassin, J P and Moyne, M A , Dopaminergic innervation of the rat prefrontal cortex a fluorescence histochemical study, *Brain Research*, 106 (1976) 133–145
8 Berk, M L and Finkelstein, J A , Efferent connections of

the lateral hypothalamic area of the rat an autoradiographic investigation, *Brain Res Bull*, 8 (1982) 511–526
9 Bielajew, C and Shizgal, P, Dissociation of the substrates for medial forebrain bundle self-stimulation and stimulation-escape using a two-electrode stimulation technique *Physiol Behav*, 25 (1980) 707–711
10 Bielajew, C and Shizgal, P, Behaviorally derived measures of conduction velocity in the substrate for rewarding medial forebrain bundle stimulation, *Brain Research*, 237 (1982) 107–119
11 Britt, M D and Wise, R A Kainic acid spares fibers of the dorsal noradrenergic bundle, *Brain Res Bull*, 7 (1981) 437–440
12 Cazala, P and Guenet, J L, Probable independence between the genetic determinants of approach and escape responses induced by lateral hypothalamic stimulation in the mouse, *Behav Neural Biol*, 37 (1983) 246–255
13 Clavier, R M and Gerfen, C R, Self-stimulation of the sulcal prefrontal cortex in the rat direct evidence for ascending dopaminergic mediation, *Neurosci Lett*, 12 (1979) 183–187
14 Corbett, D, Laterrière, A and Milner, P M, Elimination of medial prefrontal cortex self-stimulation following transection of efferents to the sulcal cortex in the rat, *Physiol Behav*, 29 (1982) 425–431
15 Deutsch, J A and Albertson, T E, Refractory period and adaptation in prolonged brain reward, *Behav Biol*, 11 (1974) 275–279
16 Deutsch, J A and Hawkins, R D, Adaptation as a cause of apparent aversiveness of prolonged rewarding brain stimulation, *Behav Biol*, 7 (1972) 285–290
17 Di Chiara, G, Morelli, M, Imperato, A, and Porceddu, M L, A re-evaluation of the role of superior colliculus in turning behaviour, *Brain Research*, 237 (1982) 61–77
18 Edwards, M, Wishik, J and Sinnamon, H M, Catecholaminergic and cholinergic agents and duration regulation of ICSS in the rat, *Pharmacol Biochem Behav*, 10 (1979) 723–731
19 Gallistel, C R, Shizgal, P and Yeomans, J S, A portrait of the substrate for self-stimulation, *Psychol Rev*, 88 (1981) 228–273
20 Gerfen, C R and Clavier, R M., Intracranial self-stimulation from the sulcal prefrontal cortex in the rat the effect of 6-hydroxydopamine or kainic acid lesions at the site of stimulation, *Brain Research*, 224 (1981) 291–304
21 Goodall, E B and Carey, R J, Effects of D- versus L-amphetamine, food deprivation, and current intensity on self-stimulation of the lateral hypothalamus, substantia nigra and medial frontal cortex of the rat, *J comp physiol Psychol*, 89 (1975) 1029–1045
22 Guldin, W O and Markowitsch, H J, No detectable remote lesions following massive intrastriatal injections of ibotenic acid, *Brain Research*, 225 (1981) 446–451
23 Hansen, S, Kohler, Ch, Goldstein, M and Steinbusch, H V M, Effects of ibotenic acid-induced neuronal degeneration in the medial preoptic area and the lateral hypothalamic area on sexual behavior in the male rat, *Brain Research*, 239 (1982) 213–232
24 Hoebel, B G, Brain-stimulation reward and aversion in relation to behavior In A Wauquier and E T Rolls (Eds), *Brain-Stimulation Reward*, North-Holland Publ Co, Amsterdam, 1976, pp 335–372
25 Hosoya, Y, The distribution of spinal projection neurons in the hypothalamus of the rat, studied with the HRP method, *Exp Brain Res*, 40 (1980) 79–87
26 Huston, J P, Ornstein, K and Lehner R The diencephalic peninsula self-stimulation after unilateral precollicular transection and removal of the telencephalon, *Brain Research*, 245 (1982) 187–191
27 Konig, J F R and Klippel, R A *The Rat Brain*, Baltimore, Williams and Wilkins, 1963
28 Krettek, J E and Price, J I The cortical projections of the medio-dorsal nucleus and adjacent thalamic nuclei in the rat, *J comp Neurol*, 171 (1977) 157–192
29 Kurumiya, S, Takeichi, T, Umemoto, M, Itakura, I and Olds, M E, Self-stimulation in regions of catecholamine hyper- or hypo-innervation produced by neonatal 6-hydroxydopamine, *Soc Neurosci Abstr*, 9 (1983) 122
30 Liebman, J M, Discriminating between reward and performance a critical review of intracranial self-stimulation methodology, *Neurosci Biobehav Rev*, 7 (1983) 45–72
31 Liebman, J M, Gerhardt S and Prowse, J Differential effects of D-amphetamine, pipradrol and bupropion on shuttle-box self-stimulation, *Pharmacol Biochem Behav* 16 (1982) 791–794
32 Liebman, J M, Hall, N and Prowse, J, Effects of various catecholamine receptor antagonists, muscle relaxation and physical hindrance on shuttle-box self-stimulation, *Pharmacol Biochem Behav*, 16 (1982) 785–790
33 Lindvall, O, Bjorklund, A and Divac, I, Organization of catecholamine neurons projecting to the frontal cortex in the rat, *Brain Research*, 142 (1978) 1–24
34 McMullen, N T and Almli, C R, Cell types within the medial forebrain bundle a Golgi study of preoptic and hypothalamic neurons in the rat, *Amer J Anat* 161 (1981) 323–340
35 Mason, S T and Fibiger, H C On the specificity of kainic acid, *Science*, 204 (1979) 1339–1341
36 Mendelson, J and Freed, W J, Do rats terminate hypothalamic stimulation only in order to turn it on again? *Behav Biol*, 8 (1973) 619–628
37 Millhouse O E, A Golgi anatomy of the rodent hypothalamus In P J Morgane and J Panksepp (Eds), *Handbook of the Hypothalamus*, M Dekker, New York, 1979, Vol 1, pp 221–265
38 Nieuwenhuys, R, Geeraedts, L M G and Veening, J G The medial forebrain bundle of the rat I General introduction, *J comp Neurol*, 206 (1982) 49–81
39 Olds, J, Hypothalamic substrates of reward, *Physiol Rev*, 42 (1962) 554–604
40 Olds, J, *Drives and Reinforcements Behavioral Studies of Hypothalamic Functions*, Raven Press New York, 1977 140 p
41 Robertson, A, Laferrière, A and Franklin, K B J, Amphetamine and increases in current intensity modulate reward in the hypothalamus and substantia nigra but not in the prefrontal cortex, *Physiol Behav*, 26 (1981) 809–813
42 Routtenberg, A, Forebrain pathways of reward in *rattus norvegicus*, *J comp physiol Psychol*, 75 (1971) 269–276
43 Routtenberg, A and Sloan, M, Self-stimulation in the frontal cortex of *rattus norvegicus*, *Behav Biol*, 7 (1972) 567–572
44 Saper, C B, Swanson, L W and Cowan, W M, An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat, *J comp Neurol*, 183 (1979) 689–706
45 Schenk, S and Shizgal, P, The substrates for lateral hypothalamic and medial pre-frontal cortex self-stimulation

have different refractory periods and show poor spatial summation, *Physiol Behav*, 28 (1982) 133–138

46 Schwarcz, R, Hokfelt, T, Fuxe, K, Jonsson, G, Goldstein, M and Terenius, L, Ibotenic acid-induced neuronal degeneration a morphological and neurochemical study, *Exp Brain Res*, 37 (1979) 199–216

47 Shizgal, P and Matthews, G, Electrical stimulation of the rat diencephalon differential effects of interrupted stimulation on ON- and OFF-responding, *Brain Research*, 129 (1977) 319–333

48 Simon, H, Stinus, L, Tassin, J L, Lavielle, S, Blanc, G, Thierry, A M, Glowinski, J and Le Moal, M, Is the dopaminergic mesocorticolimbic system necessary for intracranial self-stimulation? *Behav Neural Biol*, 27 (1979) 125–145

49 Sprick, U, Munoz, C and Huston, J P, Effect of neurotoxic lateral hypothalamic lesions on lateral hypothalamic self-stimulation, *Neurosci Lett Suppl*, 14 (1983) S354

50 Steiner, S S, Bodnar, R J, Nelson, Jr, W T, Ackermann, R F and Ellman, S J, Intracranial self-stimulation site specificity the myth of current spread, *Brain Res Bull*, 3 (1978) 349–356

51 Szabo, I, Path neuron system of medial forebrain bundle as a possible substrate for hypothalamic self-stimulation, *Physiol Behav*, 10 (1973) 315–328

52 Szabo, I, Lenard, L and Kosaras, B, Drive decay theory of self-stimulation refractory periods and axon diameters in hypothalamic reward loci, *Physiol Behav*, 12 (1974) 329–343

53 Valenstein, E S, Problems of measurement and interpretation with reinforcing brain stimulation, *Psychol Rev*, 71 (1964) 415–437

54 Valenstein, E S and Meyers, W J, Rate-independent test of reinforcing consequences of brain stimulation, *J comp physiol Psychol*, 57 (1964) 52–60

55 Velley, L and Cardo, B, Etude ontogénétique du comportement d'autostimulation chez le rat, *Physiol Behav*, 18 (1977) 183–186

56 Velley, L, Chaminade, C, Roy, M T, Kempt, E and Cardo, B, Intrinsic neurons are involved in lateral hypothalamic self-stimulation, *Brain Research*, 268 (1983) 79–86

57 Wise, R A, Catecholamine theories of reward a critical review, *Brain Research*, 152 (1978) 215–247

58 Yadin, E, Guarini, V and Gallistel, C R, Unilaterally activated systems in rats self-stimulating at sites in the medial forebrain bundle, medial prefrontal cortex or locus coeruleus, *Brain Research*, 266 (1983) 39–50