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EFFECT OF OREXIN-B-SAPORIN INDUCED LESIONS OF THE LATERAL HYPOTHALAMUS ON PERFORMANCE ON A PROGRESSIVE-RATIO SCHEDULE

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Abstract. It has been suggested that a sub-population of orexinergic neurones whose somata lie in the lateral hypothalamic area (LHA) play an important role in regulating the reinforcing value of both food and drugs. This experiment examined the effect of disruption of orexinergic mechanisms in the LHA on performance on the progressive-ratio schedule of reinforcement, in which the response requirement increases progressively for successive reinforcers. The data were analysed using a mathematical model which yields a quantitative index of reinforcer value and dissociates effects of interventions on motor and motivational processes (Killeen, 1994). Rats were trained under a progressive-ratio schedule using food-pellet reinforcement. They received bilateral injections of conjugated orexin-B-saporin (OxSap) into the LHA or sham lesions. Training continued for a further 40 sessions after surgery. Equations were fitted to the response rate data from each rat, and the parameters of the model were derived for successive blocks of 10 sessions. The OxSap lesion reduced the number of orexin-containing neurones in the LHA by approximately 50% compared to the sham-lesioned group. The parameter expressing the incentive value of the reinforcer was not significantly altered by the lesion. However, the parameter related to the maximum response rate was significantly affected, suggesting that that motor capacity was diminished in the OxSap-lesioned group. The results indicate that OxSap lesions of the LHA disrupted food-reinforced responding on the progressive-ratio schedule. It is suggested that this disruption was brought about by a change in non-motivational (motor) processes.

Keywords: orexins – orexin-saporin - operant behaviour – incentive value – motor performance – progressive ratio schedule

Introduction

Orexin A and B, also known as hypocretin 1 and 2, are neuropeptides that are expressed by groups of neurones whose somata lie in the lateral hypothalamic area (LHA) and surrounding regions (de Lecea *et al.*, 1998; Date *et al.*, 1999; Peyron *et al.*, 1998; Sakurai *et al*., 1998). Orexinergic neurones project extensively throughout the brain, and the two known types of orexin receptor (OX1 and OX2) are expressed in many brain regions (Trivedi *et al*., 1998).

The orexins have been implicated in a variety of neurobehavioural functions, including reinforcement, feeding, addiction and regulation of the sleep/wakefulness cycle (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998; de Lecea and Sutcliffe, 1999; Sakurai, 1999; Piper *et al*., 2000; Siegel, 2004, 2005; Saper *et al.*, 2005). It has been proposed that the orexinergic neurones of the hypothalamus may be divided into two anatomically distinct groups which subserve different behavioural functions ('dichotomy of orexinergic function': Harris and Aston-Jones, 2006). The first group, which consists of the orexinergic neurones of the LHA, is purported to be involved in reinforcement mechanisms. The second group, which consists of the orexinergic neurones of the dorsomedial hypothalamus (DMH) and perifornical area (PFA), is purported to be involved in the regulation of stress and arousal.

Several lines of evidence support the putative role of the LHA orexinergic projection in reinforcement mechanisms. For example, injection of orexin A into the cerebral ventricles (Sakurai *et al.*, 1998) or directly into the LHA (Dube *et al.*, 1999; Sweet *et al.*, 1999; Thorpe *et al.*, 2005) induces feeding in rodents. Consumption of high-energy diet has been found to be preferentially enhanced by orexin A (Clegg *et al.*, 2002), and the orexin receptor antagonist 1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl urea (SB-334867), administered systemically or into the ventral tegmental area (a prominent projection region of the LHA orexinergic neurones), has been found to attenuate spontaneous or opiate-induced

consumption of high-fat diet (Zheng *et al.*, 2007; Choi *et al.*, 2010). More direct evidence for an involvement of orexins in reinforcement processes derives from the finding that conditioned place preference induced by food or opiate reinforcement is associated with activation of LHA orexinergic neurones, and that conditioned place preference can be attenuated by systemic administration of SB-334867 (Harris *et al.*, 2005). Stimulation of these neurones by injection of rat pancreatic polypeptide into the LHA was found to reinstate a previously extinguished conditioned place preference for morphine, and this effect was also blocked by SB-334867 (Harris *et al.*, 2005).

There have been several reports of the effect of manipulating orexinergic function on schedule-controlled operant behaviour. Most of these studies have employed the progressive-ratio schedule of reinforcement, in which the number of responses required in order to obtain a reinforcer (the response/reinforcer ratio) is progressively increased (Hodos, 1961; Hodos and Kalman, 1963). As the ratio is increased, response rate declines, until the subject eventually stops responding, The ratio at which responding ceases for some specified period defines the 'breakpoint'. Administration of orexin A into the third ventricle (Choi *et al.*, 2010) or directly into the LHA (Thorpe *et al*., 2005) increased the breakpoint and facilitated food intake. Moreover, SB-334867 reduced the breakpoint when the reinforcer was cocaine or a highly palatable food (Nair *et al.*, 2008; Borgland *et al*., 2009; Choi *et al*., 2010; Sharf *et al.*, 2010). Since the breakpoint is often used as an index of the subject's motivational state or the incentive value of reinforcers (Hodos, 1961; Cheeta *et al.*, 1995; Ping-Teng *et al.*, 1996; Aberman *et al.*, 1998; Bowman and Brown, 1998; Barr and Phillips, 1999; Thorpe *et al.*, 2005), these findings are consistent with the notion that the LHA orexinergic projection from the LHA forms part of the neural substrate of reinforcement.

However, it has been argued that some of the findings that have been attributed to the putative role of orexinergic mechanisms in reinforcement may also be interpreted in terms of

their role in the control of muscle tone and motor output (Siegel, 2004, 2005; Berridge *et al*., 2010). Thus, Berridge *et al.* (2010) have pointed out that increased levels of arousal induced by either aversive or appetitive stimuli may facilitate the reinstatement of conditioned place preference (Stewart, 2000), and it is therefore possible that the effect of orexinergic manipulations on 'reward seeking' (Harris *et al*., 2005) may reflect reward-independent changes in arousal rather than changes in the value of positive reinforcers (Berridge *et al*., 2010).

Moreover, doubts have been raised about the validity of the breakpoint derived from progressive-ratio schedule performance as an index of reinforcer value (Arnold and Roberts, 1997; Mobini *et al.*, 2000; Ho *et al.*, 2003; Killeen *et al.*, 2009; Rickard *et al*., 2009). Although this measure is sensitive to changes in the incentive value of reinforcers (Bizo and Killeen, 1997; Rickard *et al.*, 2009), it is also affected by non-motivational variables such as the height of the operant lever (Aberman *et al*., 1998; Skjoldager *et al*., 1993) or the ratio step size (Stafford and Branch, 1998). It has also been noted that there is no clear consensus about the definition of the breakpoint (Arnold and Roberts, 1998; Killeen *et al.*, 2009).

Quantitative models of schedule-controlled behaviour provide a means of circumventing some of the problems related to the breakpoint, and may offer a more reliable basis for discriminating between the effects of neuropharmacological interventions on incentive value and motor functions. One such model is Killeen's Mathematical Principles of Reinforcement (MPR: Killeen, 1994; Killeen and Sitomer, 2001). According to this model, schedule-controlled responding is determined by three factors: a general excitatory effect of reinforcers on behaviour, a biological constraint on responding imposed by the response requirement and the physical capacities of the organism, and the efficiency with which particular reinforcement schedules couple operant responses to reinforcers. In the case of ratio schedules, in which *N* responses are required to obtain a reinforcer delivery, response

rate, *R*, is predicted by the following equation:

$$R = \frac{1-(1-\beta)^N}{\delta} - \frac{a}{N} \quad ; \qquad a, \delta > 0, \quad N \le a/\delta, \quad 0 < \beta < 1 \qquad [1]$$

The parameter β ('currency') represents the extent to which the strengthening effect of the reinforcer is focused on the most recent response; δ ('response time') expresses the constraint on responding; and *a* ('specific activation') is the time for which a reinforcer is able to activate behaviour. The function defined by Equation 1 is shown in Fig. 1. β defines the locus of the peak of the function, a reduction of β being reflected in a rightward displacement of the peak; δ defines the amplitude of the peak, an increase in δ being reflected in a reduction of the maximum response rate; and *a* defines the slope of the descending limb of the function, a lower value of this parameter being reflected in a steeper decline of response rate as a function of ratio size. The parameter *a* is purported to provide an index of reinforcer efficacy or 'value' (Reilly, 2003). In keeping with this notion, it has been found that there is a monotonic relation between reinforcer size and this parameter (Rickard *et al.*, 2009). The 'extinction ratio' is the point of intersection of the curve with the abscissa, in other words, the size of the ratio (*N*) at which the subject stops responding. This theoretical expression of the empirical breakpoint is jointly determined by *a* and δ. This implies that a change in the breakpoint may be brought about not only by a change in the incentive value of the reinforcer (*a*) but also by non-motivational factors encapsulated in δ.

Equation 1 was originally developed to describe performance on fixed- and variable-ratio schedules; however it also provides a good description of overall response rates on progressive-ratio schedules (Ho *et al.*, 2003; Killeen *et al.*, 2009; Rickard *et al.*, 2009). Application of Equation 1 to progressive-ratio schedule performance has been used successfully to detect the effects of brain lesions and drug administration on motivational and motor-related processes (Bezzina *et al.*, 2008a, 2008b; Ho *et al.*, 2003; Kheramin *et al.*, 2005; Mobini *et al.*, 2000; Zhang *et al.*, 2005a, 2005b).

A recent study of the effect of reinforcer size on progressive-ratio schedule performance (Rickard *et al.*, 2009) revealed that while Equation 1 provided a good description of overall response rate, its fit to the 'running' response rate (response rate calculated after exclusion of the post-reinforcement pause: Bizo *et al*., 2001) was less satisfactory. Running response rate was, however, well described by the logistic function:

$$R = R_i /(1+[N/b]^c) \quad [2]$$

where R_i is a parameter expressing the initial (maximum) response rate, b expresses the rate of decay of the function, and the exponent c modulates the curvature of the function. Rickard *et al.* (2009) found that b was was monotonically related to reinforcer size, while R_i was unaffected by changes in reinforcer size.

The aim of the present experiment was to provide further information about the effect of disruption of orexinergic mechanisms in the LHA on operant behaviour using a quantitative analysis of performance on the progressive-ratio schedule. Changes in the parameters of Equations 1 and 2 induced by the lesion were used to infer changes in reinforcer value and motor capacity. Disruption of orexinergic mechanisms was brought about by injection of an orexin-B-saporin conjugate (OxSap) into the LHA. This neurotoxin destroys neurones that express OX2 receptors, including orexinergic neurones, thereby disrupting the local neuronal circuitry that depends on orexinergic neurotransmission together with the orexinergic output from ths area (Gerashchenko *et al.*, 2001; see below [Discussion] for review). The effects of the lesion on locomotor behaviour, food consumption and body weight were also examined.

Methods

The experiments were carried out in accordance with UK Home Office regulations governing

experiments on living animals.

Subjects

Thirty experimentally naive female Wistar rats approximately 4 months old and weighing 250–300 g at the start of the experiment were used. They were housed under a constant cycle of 12 h light and 12 h darkness (light on 06:00 - 18:00), and were maintained at 80% of their initial free-feeding body weights throughout the operant behaviour experiment by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages.

Surgery

The rats were divided into two groups. The first group received bilateral lesions of the LHA with the orexin-B-saporin conjugate (OxSap: Advanced Targeted Systems, USA) (n=14). The control group was divided into two sub-groups which received either bilateral intra-LHA injections of unconjugated saporin (Advanced Targeted Systems, USA) (n=8), or (ii) bilateral intra-LHA injections of the vehicle solution alone (0.9% sodium chloride solution) (n=8). Anaesthesia was induced with isoflurane (4% in oxygen), and the animals were placed in a stereotaxic instrument (David Kopf), with the upper incisor bar set 3.3 mm below the inter-aural line. Isoflurane was kept at 2% in oxygen during the surgical procedure. A small hole was drilled through the skull overlying each cerebral hemisphere for microinjection of the solutions into the LHA. The following coordinates were used to locate the LHA: AP -3.3, L ± 1.6, V -9.1, measured from bregma (Paxinos and Watson, 1998).

Microinjections were administered with a 5 µl Hamilton syringe connected by a polyethylene tube to a 0.3-mm diameter cannula. The solution administered to the first group contained 15 ng OxSap dissolved in 0.3 µl nonpyrogenic 0.9 % sodium chloride (Baxter

Health Care, UK). (The dose used was based in preliminary studies carried out in this laboratory in which it was found that doses of 180 ng, 90 ng and 45 ng of OxSap produced substantial tissue damage of the LHA and surrounding areas. Furthermore these doses produced significant reductions of food intake and loss of body weight which necessitated euthanasia of most animals 2-3 weeks after surgery.) The cannula was slowly lowered to the target position, and the solution was injected during a 60-s period. The cannula was left in position for 3 minutes after completion of the injection, and was then withdrawn. The procedure for the other two groups was identical except that the injections were either 15 ng unconjugated saporin in 0.3 μl saline (n=8) or 0.3 μl of the saline solution (n=8).

Apparatus

Operant behaviour chambers. The rats were trained in custom built operant conditioning chambers of internal dimensions 20 cm × 23 cm × 22.5 cm. One wall of the chamber contained a recess into which a motor-operated dispenser could deliver 45-mg food pellets (TestDiet, MLab Rodent Tablet 45 mg; Sandown Scientific, UK). An aperture was situated 5 cm above and 2.5 cm to the left of the recess, through which a motor-operated retractable lever could be inserted into the chamber. The lever could be depressed by a force of approximately 0.2 N. The chamber was enclosed in a sound-attenuating chest; masking noise was provided by a rotary fan. An Acorn 5000 microcomputer and interface unit (Paul Fray Ltd.), programmed in ARACHNID BASIC and located in an adjoining room, controlled the schedules and recorded the behavioural data.

Locomotor behaviour chambers. Locomotor behaviour was recorded in transparent Perspex boxes of internal dimensions 42 cm × 32 cm × 29 cm. Each box was equipped with eight infrared photocell beams located at equal intervals along the long axis of the box, 4 cm above the floor. An Acorn microcomputer programmed in ARACHNID BASIC and located

in an adjoining room, recorded the data.

Behavioural procedures

Progressive ratio schedule. Two weeks before the start of behavioural training, the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. Then they were trained to press the lever for a food-pellet reinforcer (45 mg) and were exposed to a fixed-ratio 1 schedule for 3 sessions, followed by fixed-ratio 5 for a futher 3 sessions. Thereafter, they underwent daily training sessions under the progressive-ratio schedule. The progressive-ratio schedule was based on the following exponential progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, . . . , derived from the formula $[(5\times e^{0.2n})-5]$, rounded to the nearest integer, where n is the position in the sequence of ratios (Roberts and Richardson, 1992). Sessions took place at the same time each day during the light phase of the daily cycle (between 07:00 and 14:00) 7 days a week for the duration of the experiment. At the start of each session, the lever was inserted into the chamber; the session was terminated by withdrawal of the lever 50 min later. The rats were trained under the progressive-ratio schedule until the estimated parameters of Equation 1 (see *Data analysis*) had attained stability according to the following criterion. Each parameter was deemed to be stable when the cumulated change in the group mean value of the parameter over three successive blocks of 10 sessions was $\leq$ 10%. The value of parameter a increased steadily over 60 sessions and the formal criterion was not met until the 10^{th} block. Surgery was carried out after 110 training sessions. Training continued for 40 sessions after surgery.

Locomotor behaviour. Each rat was tested at the same time of day, and under the same food restriction conditions as in the operant behaviour experiment. Each session was 30 min in duration and the data were recorded in 6 successive epochs of 5 min. The tests took place 23 h after the last meal. The rats underwent the locomotor activity testing on 7

successive days: 5 habituation sessions and two recording sessions.

Food intake. Food intake tests took place in the animal holding room. Each rat was placed in a cage similar to the home cage containing a pre-weighed dish containing approximately 25 g of the same 45-mg food pellets as were used in the operant behaviour experiment. The rats were left undisturbed for 60 min before being returned to their home cages. The dishes were re-weighed and the weight of food consumed was calculated. Three test sessions were carried out at intervals of 48 h while the rats were maintained under the same food deprivation conditions as in the operant behaviour experiment. In a second phase, the same procedure was carried out while the rats had free access to standard chow in their home cages. In this phase, the amount of chow consumed in 23 hours was also measured.

Immunohistochemistry

At the end of the behavioural experiment, the rats were deeply anaesthetised with sodium pentobarbitone, and perfused transcardially with phosphate-buffered sodium chloride solution (PBS), followed by 10% formol PBS. The brains were removed from the skull and fixed in formol PBS for 3 days. Coronal sections (60-µm) were taken through the LHA region using a freezing microtome. Alternate sections were selected for labelling of neurone-specific nuclear protein (NeuN) and orexin.

NeuN was labelled as described by Jongen-Relo and Feldon (2002). The protocol followed was described by Bezzina et al. (2007). Three freshly sliced sections, taken at approximately 120-µm intervals between AP +3.0 and AP +3.6 were washed in 0.1 M PBS and placed in 0.5% H_2O_2 in PBS for 30 min at room temperature. Then they were washed twice in PBS and placed for 1 h in a blocking solution [10% normal horse serum (Vector Laboratories, Peterborough, UK), 1% bovine serum albumin (BSA, Sigma-Aldrich, Gillingham, UK) and 0.3% Triton X-100 (Sigma-Aldrich) in PBS]. They were then incubated

for 48 h at 4°C in the primary antibody [monoclonal mouse anti-NeuN serum (1:5,000, Chemicon, Chandlers Ford, UK) in 1% normal horse serum, 1% BSA and 0.3% Triton X-100 in PBS], washed twice in PBS, and incubated for 2 h at room temperature in biotinylated horse antimouse serum (Vector Laboratories; 1:1,000 in 1% BSA and 0.3% Triton X-100 in PBS). After two washes in PBS, they were placed for 2 h in avidin–biotin–horseradish peroxidase complex (1:200, ABC-Elite, Vector Laboratories) in PBS. Following two further washes in PBS, they were placed in a chromagen solution [0.05% diaminobenzidine (Sigma-Aldrich) and 0.01% H_2O_2 (Sigma-Aldrich)] for 5 min. The reaction was observed visually and stopped by washing in PBS. The sections were floated on to chrome-gelatine-coated slides and mounted with DPX.

The procedure for orexin staining was the same as that described above, with the exception that the primary antibody was goat anti-orexin diluted 1:5,000 in PBS (Santa Cruz Biotechnology, USA) and the secondary antibody was horse anti-goat serum 1:1000.

NeuN-positive nuclei and orexin-positive neurones were counted blind from coded images of the sections using Image-J software (Wayne Rasband, National Institutes of Health, USA) .

Data analysis

The data from one rat belonging to the sham lesioned group were discarded, because this rat failed to complete a sufficient number of ratios in each session to permit curve fitting analysis to be performed. A preliminary analysis comparing the immunohistochemical data and post-surgical performances of the two control sub-groups (i.e. the rats that received intra-LHA injections of unconjugated saporin and the saline vehicle) revealed no significant differences between the numbers of orexin-positive neurones or NeuN-positive nuclei or any of the performance measures, and therefore the data from the two sub-groups were pooled in all

subsequent analyses. Analyses were thus based on the data from the OxSap-lesioned group (n=14) and the combined sham-lesioned group (n=15).

Operant behaviour data. Data from the last block of 10 sessions before surgery and the four successive blocks of 10 sessions following surgery were used in the analyses.

Peak response rate. Differences between the highest overall response rate (see below) attained during performance of the progressive-ratio schedule during the final pre-surgical block of sessions and the four post-surgical blocks were analysed by two-factor analysis of variance (group × block) with repeated measures on the latter factor. In the case of a significant main effect of group or a significant interaction term, comparisons were made between groups at each block using the least significance difference test.

Highest completed ratio and breakpoint. The breakpoint was defined as the last ratio to be completed before 5 min elapsed without any responding (Hodos 1961; Hodos and Kalman 1963). In most cases, this was identical to the highest ratio completed in the session. However, in some cases, this criterion was not met within the 50-min session. Therefore, the highest completed ratio and the breakpoint were analysed separately. The data were analysed in the same way as the peak response rate. Analysis of the highest completed ratio incorporated the data from all sessions, whereas analysis of the breakpoint entailed exclusion of data from sessions in which the formal breakpoint criterion was not attained.

Overall response rate. Overall response rate was calculated for each ratio using the total time taken to complete the ratio, including the post-reinforcement pause, measured from the end of the preceding reinforcer delivery until the emission of the last response of the ratio (Bizo and Killeen, 1997). The first ratio (a single response) and any ratios that had not been completed at the end of the session were excluded from the analysis. The raw data from the final pre-surgical and the four post-surgical blocks of 10 sessions were analysed by three-factor analysis of variance (group × block × ratio) with repeated measures on the second and

third factors. Equation 1 was fitted to the overall response rate data from each rat using an iterative least-squares method (SigmaPlot, Version 8.0), and the estimated values of the parameters β, δ and *a* were derived; goodness of fit was expressed as r^2, the proportion of the data variance accounted for by the equation. In agreement with previous findings (Mobini *et al.*, 2000; Ho *et al.*, 2003; Zhang *et al.*, 2005a, 2005b; Bezzina *et al.*, 2008a, 2008b; Rickard *et al.*, 2009), examination of the data revealed that in some rats very low response rates were generated under the highest ratios, which did not conform to Equation 1. Therefore the equation was fitted to each rat's data after exclusion of these low rates using the following operational criterion (Mobini *et al.*, 2000). Points were removed successively, starting from the highest ratio completed, when the curve-fitting routine generated an abscissa intersection point (a/δ) which lay to the left of the rightmost empirical datum point; such an intersection implies a negative predicted response rate, which is impossible empirically, and specifically precluded by the model (see above, Equation 1). A fit was accepted when the predicted response rates for all the surviving data points had positive values. This procedure seldom eliminated more than one datum point from the data sets derived from individual rats. A preliminary analysis of the estimates of the three parameters of Equation 1 (a, δ and β) in successive blocks of 10 sessions before surgery (analysis of variance: group × block) was carried out in order to check that there were no significant between-group differences in the pre-surgical data. Then the post-surgical changes of the estimates of the three parameters obtained during the final pre-surgical block of sessions and the four post-surgical blocks were analysed in the same way as the highest completed ratio and peak response rate. An additional analysis comparing the fits of Equation 1 to data from the pre-surgical and final post-surgical blocks in each group was carried out with non-linear mixed effect modelling using the maximum likelihood approach (Pinheiro and Bates 2004).

Running response rate. Running rate was calculated by dividing the number of responses by the 'run-time' (i.e. the time taken to complete the ratio, excluding the post-reinforcement pause: Bizo *et al.*, 2001). The data were analysed as described above. Equation 2 was fitted to the running rate data from the individual rats and post-surgical changes in the parameters were analysed in the same way as the parameters of Equation 1.

Because the number of ratios completed within a session under a progressive-ratio schedule differs among individual subjects, analyses of variance of the raw response rates included only those ratios that were completed by at least 75% of the rats in each group in each phase of the experiment (ratios up to and including 62), missing values being filled using the value obtained in the highest ratio completed by the subject in question (Rickard *et al*., 2009). (Note that this limitation did not apply to the quantitative analysis of response rates using Equations 1 and 2, which entailed fitting functions to the data from individual rats.)

Locomotor activity data. Data were averaged across the two test sessions. The total numbers of beam breaks recorded in successive 5-min epochs were analysed by two-factor analysis of variance (group × epoch) with repeated measures on the latter factor.

Food intake data. Comparisons between groups were made using Student's *t*-test.

A significance criterion of $p \leq 0.05$ was adopted in all statistical analyses.

Results

Operant behaviour data

Peak response rate. The mean (±SEM) post-/pre-surgical differences in peak response rate for the sham-lesioned and the OxSap-lesioned group are shown in Fig. 2 (left-hand panel). Analysis of variance revealed no significant main effects of group [$F(1,27)$ =

2.24, *NS*] or block [$F<1$]. However there was a significant group × block interaction [$F(3,81) = 4.26$, $p<0.01$], reflecting a decrement of the maximum response rate in the OxSap-lesioned group in the last three blocks of sessions after surgery.

Highest completed ratio and breakpoint. Both the highest completed ratio (Fig. 2, middle panel) and the breakpoint (Fig. 2, right-hand panel) were somewhat reduced after surgery, the reduction appearing slightly greater in the OxSap-lesioned group than in the sham-lesioned group. However, analysis of variance of the highest completed ratio revealed no significant main effect of group [$F<1$] or block [$F(3,81) = 1.05$, *NS*] and no significant interaction [$F<1$]. In the case of the breakpoint, there was a significant effect of block [$F(3,69) = 3.0$, $p<0.05$], but no significant effect of group and no significant interaction [*F*s<1].

Overall response rate. Fig. 3 shows the estimates of the parameters of Equation 1 in successive blocks of 10 sessions in the pre-surgical phase of the experiment. The parameter *a* showed a progressive increase during training, and the formal stability criterion (see above: *Method*) was not attained until the 10th block of sessions. Analysis of variance revealed a significant main effect of block [$F(10,270) = 10.9$, $p<0.001$], but no significant effect of group and no significant interaction [*F*s<1]; moreover there was no significant difference between the groups in the final pre-surgical block [$t(27) = 0.6$, *NS*]. The other two parameters (δ and β) remained relatively stable during the training phase, there being no significant differences between the two groups across the eleven blocks [all *F*s<1.2, *NS*] nor any significant difference in the final-pre-surgical block. [δ: $t(27) = 0.8$, *NS*; β: $t(27) = 0.5$, *NS*] Fig. 4 shows the group mean overall response rate data for the sham-lesioned (left hand panel) and OxSap-lesioned groups (right hand panel) in the last pre- and post-surgical blocks of sessions. In both groups, response rate increased rapidly to a peak, and then gradually declined as the response/reinforcer ratio was progressively increased. The maximum response

rate was reduced in the Ox-Sap lesioned group following surgery. The fits of Equation 1 to the group mean data accounted for >90% of the total variance (*sham lesion*: pre-surgery, r^2 = 0.93; post-surgery, r^2 = 0.90; *OxSap lesion*: pre-surgery, r^2 = 0.94; post-surgery, r^2 = 0.92). There were significant main effects of block [F (4,108) = 6.2, p<0.001] and ratio [F(11,297) = 20.1, p<0.001], but not of group [F<1]. There were significant group × block [F(4,108) = 4.5, p<0.01] and block × ratio [F(44,1188) = 1.9, p<0.01] interactions; the group × ratio and three-way interactions were not significant [Fs<1].

Fig. 5 shows the changes in the parameters of Equation 1 following surgery.

'Specific activation', *a*. There was no significant main effect of group [F<1] or block [F(3,81) = 1.1, *NS*], and no significant group × block interaction [F<1].

'Response time', δ. There was a significant main effect of group [F(1,27) = 5.6, p<0.05], but not of block [F(3,81) = 2.1, *NS*]. There was a significant group × block interaction [F(3,81) = 2.3, p<0.05]. Multiple comparisons indicated that there was a significant increase in δ in the OxSap-lesioned group, compared to the sham-lesioned group in the second, third and fourth post-surgical blocks.

'Currency', β. There was no significant main effect of group [F<1] or block [F(3,81) = 1.5, *NS*], and no significant interaction [F (3,81) = 1.3, *NS*].

The fits of Equation 1 to data from the final pre-surgical and post-surgical blocks were also analysed by non-linear mixed effect modelling using the maximum likelihood approach (see Pinheiro and Bates 2004). First, a fixed effect of block was not included in the model (model i). Then the model was modified to incorporate block as a fixed effect (model ii). In the case of the sham-lesioned group, it was found that block did not account significantly for the variability in either *a* [t(443) = 1.85, *NS*] or δ [t(443) = 1.3, *NS*], and comparison of models i and ii indicated no advantage of including block as a fixed effect [Akaike Information Criterion (AIC): model i, 4091.5; model ii, 4091.8; Likelihood Ratio

(LR) = 3.7, *NS*]. In the case of the OxSap-lesioned group, there was no significant effect of block on the estimate of *a* [$t(424) = 1.3$, *NS*]; however block exerted a significany influence on the estimate of δ [$t(424) = 12.9$, $p<0.001$]. A version of the model that incorporated an effect of block on δ but not on *a* (model iii) provided a better account of the data than the simpler model i [AIC: model i, 4033.8; model iii, 3801.3; LR = 234.5, $p<0.001$]; addition of the fixed effect of block on *a* (model ii) conferred no further advantage [AIC: model ii, 3802.5; model ii *versus* model iii: LR = 0.8, *NS*].

Running response rate. Fig. 6 shows the group mean running response rate data for the sham-lesioned (left-hand panel) and OxSap-lesioned groups (right-hand panel) in the last pre- and post-surgical blocks of sessions. In both groups, running rate declined monotonically as a function of the ratio requirement. Running rates were reduced following surgery in the OxSap-lesioned group. The fits of Equation 2 to the group mean data accounted for about 95% of the total variance. There were significant main effects of block [$F(4,108) = 7.2$, $p<0.001$] and ratio [$F(11,297) = 115.2$, $p<0.001$], but not of group [$F<1$]. There was a significant group × block [$F(4,108) = 10.0$, $p<0.01$] interaction; the block × ratio [$F(44,1188) = 1.2$, *NS*], group × ratio [$F<1$] and three-way interactions [$F<1$] were not significant.

Fig. 7 shows the changes in the parameters of Equation 2 following surgery.

'Initial running rate', R_i. There was a significant main effect of group [$F(1,27) = 4.9$, $p<0.05$], reflecting a lower value of this parameter in the OxSap-lesioned group than in the sham-lesioned group. The main effect of block [$F<1$] and the interaction [$F(3,81) = 1.7$, *NS*], were not significant.

'Decay parameter', *b*. There was no significant main effect of group [$F<1$] or block [$F<1$], and no significant interaction [$F(3,81) =1.2$, *NS*].

'Exponent', *c*. There was no significant main effect of group [$F(1,27) = 2.7$, *NS*] or block [$F<1$], and no significant interaction [$F<1$].

Locomotor activity data

Fig. 8 shows the locomotor activity data in successive 5-minute epochs of the 30-min test sessions. There was a significant main effect of epoch [$F(5,135) = 18.9$, $p<0.001$], reflecting the decline in activity after the first epoch. There was no significant main effect of group or group × epoch interaction [Fs<1].

Food intake and body weight

Feeding tests. Table 1 shows the total weights of 45-mg food pellets consumed during the 60-min tests under the food-restricted and free-feeding conditions. In neither case did food consumption differ between the two groups [t<1 in each case]. Consumption of standard chow during the 23 hours between feeding tests under the free-feeding condition did not differ between the groups [$t(27) = 1.0$, *NS*].

Body weight. During the operant behaviour experiment, the rats' body weights were maintained at 80% of their initial free-feeding weights. There was no significant difference between the weights of the food rations needed to maintain the criterion weights in the two groups [group × blocks of sessions: all Fs<1]. On return to the free-feeding condition, the body weights of both groups rapidly increased to their free-feeding levels; there was no significant difference between the two groups [$t(27) = 1.5$, *NS*; see Table 1].

Immunohistochemistry

Fig. 9 shows representative photomicrographs of sections stained for orexin (upper panels) and NeuN (lower panels). OxSap resulted in a marked loss of orexin-positive neurones from the LHA with a smaller loss from the perifornical region. Numerical data are shown in Fig. 10. There was a significant reduction of the number of orexin-positive

neurones in the LHA [approximately 50% reduction: $t(27) = 3.7$, $p<0.001$], whereas the reduction fell short of statistical significance in the case of the perifornical area [$t(27) = 1.5$, *NS*]. There was a small reduction of the number of NeuN-positive nuclei in the LHA in the lesioned group [approximately 12% reduction: $t(27) = 1.9$, $p = 0.06$]; there was no significant difference between the two groups in the case of the perifornical area [$t<1$].

Discussion

Injection of the OxSap neurotoxin into the LHA produced a significant depletion of orexin-containing neurones from the LHA. The lesion was most pronounced in the LHA. Some loss of orexinergic neurones was evident in the perifornical region in some rats; however, this was not statistically significant in the group as a whole. The destruction of orexinergic neurones by local injection of OxSap into the LHA is consistent with previous reports (Gerashchenko *et al.*, 2001, 2003; Blanco-Centurion *et al*., 2007; Furlong and Carrive, 2007; Frederick-Duus *et al.*, 2007; Vetrivelan *et al.*, 2009; Anaclet *et al.*, 2010; Di Sebasiano *et al.*, 2010, 2011). OxSap, a conjugate of orexin-B with the ribosome-inactivating protein saporin, acts by binding to OX2 orexin receptors, thereby destroying neurones that express these receptors. Since orexinergic neurones possess OX2 receptors, these neurones fall victim to the neurotoxin. However, some other neuronal populations within in the LHA also possess OX2 receptors (see below), and it is likely, therefore, that these other neurones were also affected by the lesion.

It is well established that orexinergic neurones of the LHA are susceptible to the neurotoxic effects of OxSap; however the relative vulnerability of other neuronal phenotypes remains controversial. In an early study employing OxSap, Gerashchenko *et al.* (2001) found that in addition to orexin-containing neurones, melanocortin concentrating hormone (MCH)-

containing neurones were also destroyed by OxSap, whereas neurones expressing *a*-melanocyte-stimulating hormone were resiliant. The susceptibility of MCH-containing neurones to destruction by OxSap has been confirmed in some subsequent studies (Furlong and Carrive, 2007; Ocampo-Garces *et al.*, 2011). However, there is evidence that MCH-containing neurones may be less vulnerable than orexin-containing neurones (Frederick-Duus *et al.*, 2007), and indeed some authors found that injections of OxSap into the LHA which produced profound loss of orexinergic neurones had no significant effect on the numbers of MCH-containing neurones in this area (Di Sebastiano *et al.*, 2010, 2011). It is generally agreed that hypothalamic histaminergic neurones are sensitive to OxSap (Blanco-Centurion *et al.*, 2007; Luo and Leung, 2010), as are cholinergic neurones on the lateral dorsal tegmentum (Blanco-Centurion *et al.*, 2004). Interestingly, noradrenergic neurones of the locus coeruleus have been found to be resistant to OxSap, probably reflecting the fact that these orexin-sensitive neurones express OX1 rather than OX2 receptors (Blanco-Centurion *et al.*, 2004).

The relative selectivity of OxSap for OX2 receptor-bearing neurones appears to be dose-dependent. High doses of OxSap generally produce non-selective neuronal loss (Mistlberger *et al.*, 2003; Gerashchenko *et al.*, 2006; Furlong and Carrive, 2007), whereas the effect of lower doses tends to be more selective (Gerashchenko *et al.*, 2006; Vetrivelan *et al.*, 2009; Anaclet *et al.*, 2010). In the present experiment, the number of neurones destroyed by OxSap appears to have been a relatively small proportion of the total population of neurones in the affected region, as indicated by the modest reduction of the number of NeuN-positive nuclei seen in the OxSap-lesioned group compared to the sham-lesioned group (relative loss, 12%; p=0.06). This is consistent with the results of some previous studies which employed low doses of OxSap (Gerashchenko *et al.*, 2006; Di Sebastiano *et al.*, 2010, 2011). Therefore, taking all these factors into account, we suggest that while the lesion used in this experiment produced a substantial depletion of orexinergic neurones from the LHA and presumably

induced a major disruption of orexinergic output from this area, it is not possible to quantify the contribution of a putative loss of other orexin (OX2) receptor-bearing neurones of the LHA to the behavioural effects of the lesion.

The performance of both the sham-lesioned and OxSap-lesioned groups on the progressive-ratio schedule was similar to that seen in many previous studies (Bizo and Killeen, 1997; Mobini *et al.*, 2000a; Ho *et al.*, 2003; Kheramin *et al.*, 2005; Zhang *et al.*, 2005a, 2005b; Bezzina *et al.*, 2008a, 2008b; Killeen *et al.*, 2009; Rickard *et al.*, 2009). Overall response rate was bitonically related to ratio size; it increased rapidly to reach a peak, and then declined gradually as the response/reinforcer ratio was progressively increased. The running response rate declined monotonically as a function of the ratio size. In agreement with these previous studies, overall response rates on the progressive-ratio schedule were well described by Equation 1 and running rates by Equation 2.

It has been proposed that orexinergic neurones of the LHA play an important role in reinforcement processes and that, consequently, inactivation of these neurones reduces the rewarding value of food and other reinforcers (Harris *et al.*, 2005; Harris and Aston-Jones, 2006; Aston-Jones *et al.*, 2009; Cason *et al.*, 2010; Sharf *et al.*, 2010). As discussed above, the progressive-ratio schedule has been widely used as a method for evaluating the efficacy of reinforcers (see Introduction for references). It was therefore expected that destruction of orexinergic neurones of the LHA would suppress responding on this schedule. However, Killeen's (1994) theoretical analysis of reinforcement schedules indicates that suppression of responding on ratio schedules may be caused not only by a reduction of reinforcer efficacy (represented by the parameter a) but also by an increase of the time required to emit an operant response (represented by the parameter δ). A change in either or both of these parameters is expected to alter the traditional index of progressive-ratio schedule performance, the breakpoint.

In contrast to the parameters of Equation 1, which are derived from analysis of the response rates generated by the entire sequence of ratios in the schedule, the breakpoint is defined by a single point, the ratio at which responding ceases for some predefined period (see Introduction). As time-constrained sessions were employed in this experiment, the breakpoint criterion was not achieved by all rats in all sessions, and analysis of the breakpoint data excluded those sessions in which the breakpoint criterion was not met. As an additional measure, which did not entail exclusion of data, the highest ratio completed in the session was also analysed (Zhang *et al.*, 2005a, 2005b; Rickard *et al.*, 2009). Although both measures showed a tendency to to be reduced following the OxSap lesion, the change was not statistically significant in either case.

Application of Equation 1 to the overall response rate data indicated that the OxSap lesion had no significant effect on *a*, suggesting that the lesion did not alter the intrinsic value of the reinforcer. This conclusion is supported by the analysis of the running rate data using Equation 2, which showed that the decay parameter, *b*, which has recently been shown to be systematically related to reinforcer value (Rickard *et al.*, 2009), was not significantly altered.

On the other hand, the OxSap lesion produced a substantial increase in the 'response time' parameter, δ, of Equation 1, a reduction of the 'initial response rate' parameter, R_i, of Equation 2, and a reduction of the peak response rate. Since δ specifies the minimum time required to execute an operant response, the increase of this parameter in the OxSap-lesioned group is consistent with an impairment of motor capacity. The reduction of R_i is also consistent with this interpretation, and it is noteworthy that, unlike δ, R_i is not influenced by the duration of the post-reinforcement pause; therefore the reduction of this parameter cannot readily be attributed to subtle changes in post-prandial behaviour in the lesioned group (see Bezzina *et al.*, 2008a, 2008b; Rickard *et al.*, 2009). R_i has been found to be impervious to changes in reinforcer magnitude (Rickard *et al.*, 2009), which further argues against the

possibility that the effect of the lesion might have been caused by a change in reinforcer value.

An impairment of motor function induced by the OxSap lesion is consistent with previous reports that stimulation of orexinergic mechanisms increases, and suppression of orexinergic mechanisms reduces, postural muscle tone and motor output (Ida *et al.*, 1999; Hara *et al.*, 2001; Jones *et al.*, 2001; Kiyashchenko *et al*., 2001; Wu *et al.*, 2002; Torterolo *et al.*, 2003; Takakusaki, 2008; Anaclet *et al.*, 2009).

The lack of effect of the lesion on the parameters of Equations 1 and 2 that are related to the incentive value of reinforcers is in apparent disagreement with previous findings that are consistent with the notion that the LHA orexinergic projection is critically involved in determining reinforcer efficacy. Evidence consistent with this proposition derives from a range of pharmacological manipulations (e.g., systemic or central administration of orexin receptor antagonists: Clegg *et al.*, 2002; Harris *et al.*, 2005, 2006; Zheng *et al.*, 2007; Borgland *et al.*, 2009; central administration of orexin or its precursor: Thorpe and Kotz, 2005; Thorpe *et al.*, 2005; Choi *et al.*., 2010), and a variety of behavioural paradigms (e.g., conditioned place preference: Harris *et al.*, 2005, 2007; Narita *et al.*, 2006; consumption of palatable diet: Clegg *et al.*, 2002; Zheng *et al.*, 2007; Nair *et al.*, 2008; Borgland *et al.*, 2009; Sharf *et al.*, 2010; performance on progressive-ratio schedules: Thorpe *et al.*, 2005; Borgland *et al.*, 2009; Choi *et al.*, 2010). Several methodological factors may have contributed to the apparent discrepancy between the present findings and the results of previous experiments. Firstly, it may be noted that no previous investigation of the effect of manipulating orexinergic function on reinforcement processes has used the neurotoxin OxSap to destroy LHA orexinergic neurones. In the present experiment, OxSap injected into the LHA induced a 50% loss of orexin-containing neurones from this area. It is possible that a more extensive depletion may be required in order to reveal an effect on the incentive value of food

reinforcers.

Another potentially important factor is the level of food deprivation. The subjects of this experiment were maintained at 80% of their free-feeding body weights, whereas most previous studies of the effect of manipulating orexinergic function on motivated behaviour have been carried out on non-deprived animals or under milder deprivation conditions than that used in this experiment (Thorpe *et al.*, 2005; Harris and Aston-Jones, 2006; Borgland *et al.*, 2009; Cason *et al.*, 2010; Sharf *et al.*, 2010). It is not clear how this might have influenced the results, although the control of food intake is known to be different in non-deprived and deprived states (Rowland *et al.*, 2001). It is known that in food-deprived subjects various pathways in addition to those involving orexinergic mechanisms modulate food intake. For example, food deprivation increases immediate-early gene expression in neuropeptide-Y containing neurones of the arcuate nucleus (Cummings *et al.*, 2001), and plasma ghrelin is elevated by food deprivation (Gerard *et al.*, 1990; Meister, 2007). Conceivably, the operation of non-orexinergic mechanisms might have compensated for an effect of orexin depletion on reinforcer value in the food-deprived animals used in the present study. In this context, it is of interest that Sharf *et al.* (2010) recently found that the performance of mice lacking the orexin precursor prepro-orexin (orexin -/-) on a progressive-ratio schedule did not differ from that of normal mice. Sharf *et al.* (2010) suggested that non-orexinergic mechanisms may have compensated for the lack of orexin in the genetically modified animals.

Yet another potentially relevant methodological factor is the length of training which the rats underwent before surgery. In most previous experiments in which the progressive-ratio schedule has been used to evaluate the effects of manipulating orexinergic function, the initial training period has been in the order of 5-15 sessions (Thorpe *et al.*, 2005; Borgland *et al.*, 2009; Choi *et al.*, 2010). Although responding on the progressive-ratio schedule is

generally well established within a few days of training, we have found that many sessions are required in order to attain stability of the parameters of Equation 1 (Kheramin *et al.*, 2005; Zhang *et al.*, 2005a, 2005b; Bezzina *et al.*, 2008a, 2008b; Rickard *et al.*, 2009). In the present experiment, attainment of the formal stability criterion based on the parameters of Equation 1 required approximately 100 training sessions; this was especially apparent in the case of the 'specific activation' parameter, *a* (see Figure 3). It is not clear how such protracted training might have affected the results of this experiment. There is evidence that after extended training learned behaviour tends to become 'habitual' and relatively insensitive to reinforcer devaluation or changes in motivational level (Dickinson, 1985; Miles *et al.*, 2003; de Wit *et al.*, 2010). It is possible that our rats' extensive experience with the progressive-ratio schedule reduced the impact of an OxSap-induced decrease in reinforcer value on schedule performance. Further experiments may be warranted to examine the impact of prior training on the sensitivity of operant behaviour to manipulation of orexinergic function.

The 'currency' parameter, β, of Equation 1 did not differ significantly between the two groups. β expresses the 'coupling' of responding to reinforcers, which is purported to be determined, in part, by the working memory limitations of the organism (Killeen, 1994). An effect of the OxSap lesion on this parameter might therefore have been expected, in view of evidence indicating that manipulation of orexinergic function in the medial septum, hippocampus and dentate gyrus, whose orexinergic afferents emanate from the LHA, can alter mnemonic functions, including working memory (Smith and Pang, 2005; Akbari *et al.*, 2006, 2008). However, it has yet to be demonstrated experimentally that the empirical value of β derived from ratio schedule performance is sensitive to disruption of working memory.

There was no significant difference between the spontaneous locomotor activity of the OxSap-lesioned and sham-lesioned rats in this experiment. Previous studies have shown that

acute central administration of orexins increases spontaneous locomotor behaviour, whereas orexin receptor antagonists reduce it (Jones *et al.*, 2001; Thorpe and Kotz, 2005). However, orexin deficient rodents have been found to show normal levels of locomotor activity (Sharf *et al.*, 2010) or altered activity levels that are restricted to a brief period at the start of the dark phase of the daily cycle (Hara *et al.*, 2001). It is possible that alteration of locomotor behaviour is not sustained on a chronic basis in the absence of orexinergic function. In this experiment the rats underwent locomotor testing approximately six weeks after the OxSap lesion had been inflicted. It would be of interest to examine whether locomotor behaviour is suppressed at an earlier stage following this lesion.

Food intake was not affected by the OxSap lesion in this experiment. Previous reports have emphasised the preferential effect of orexinergic manipulations on the ingestion of especially palatable foods in non-deprived animals (Sakurai *et al.*, 1998; Farr *et al*., 2005; Thorpe *et al*., 2005; Thorpe *et al*., 2006; Thorpe and Kotz, 2005; Nair *et al*., 2008; Borgland *et al*., 2009). The food pellets used in the food intake tests in the present experiment were the same as those used in the operant behaviour tests. Although these pellets are more palatable than standard laboratory chow, it is possible that they were not sufficiently appetizing to reveal an effect of the lesion on food intake.

In conclusion, the results obtained in this experiment confirm that disruption of orexinergic mechanisms in the LHA results in a significant disruption of food-reinforced operant behaviour maintained under the progressive-ratio schedule. Quantitative analysis of performance based on Killeen's (1994) mathematical model of schedule-controlled behaviour (MPR) indicated that this disruption was not brought about by a change in the incentive value of the food reinforcer, but by a change in non-motivational processes that are encapsulated in the 'response-time' parameter, δ, of Killeen's (1994) model. This is consistent with the suggestion that motor effects may play a more important role in the effects of manipulating

orexinergic function on reinforced behaviour than has previously been recognised (Siegel, 2004, 2005; Berridge *et al.*, 2010).

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Table 1. Results of food consumption tests and body weight

	Sham-lesioned group	OxSap-lesioned group
Consumption of food pellets (g per 60-min session)		
Food-restricted condition	12.8 ± 0.4	13.2 ± 0.5
Free-feeding condition	5.6 ± 0.5	6.1 ± 0.4
Home-cage chow consumption (g per 23 h)		
Free-feeding condition	22.4 ± 0.6	23.7 ± 1.1
Body weight (g)		
Free-feeding condition	277.8 ± 2.6	283.0 ± 2.7

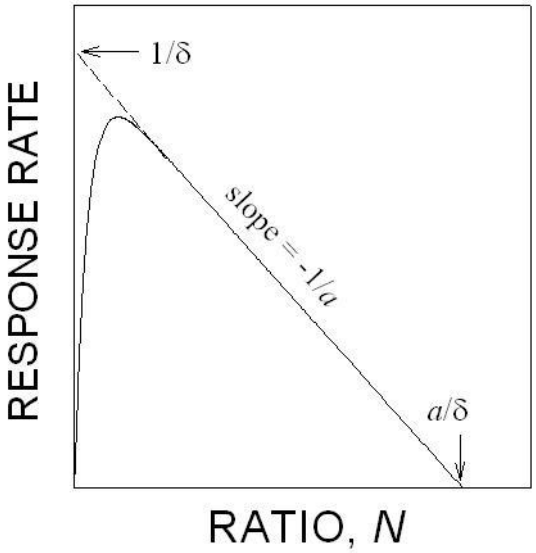

Figure 1. Theoretical function defined by Equation 1 (Killeen 1994). *Ordinate*: response rate; *abscissa*: response/reinforcer ratio (*N*). The (projected) point of intersection of the function with the ordinate is at $R = 1/\delta$, and the slope of the descending limb of the function is $-1/a$; the point of intersection with the abscissa ('extinction ratio') is jointly determined by δ and a ($N = a/\delta$). See text for details.

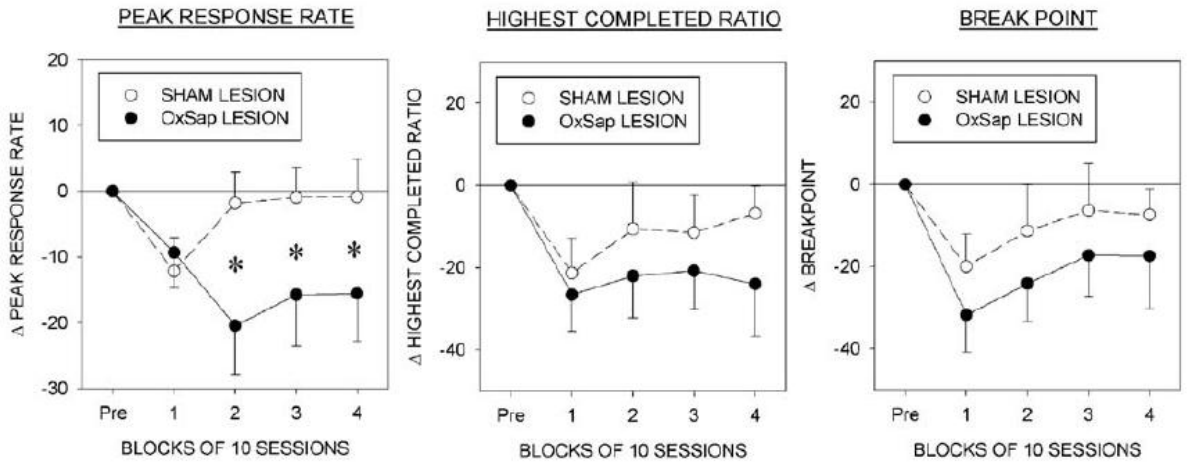

Figure 2. *Left-hand panel*. Changes in peak response rate following surgery in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups. *Ordinate*: change in the peak response rate (responses min^{-1}); *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p<0.05$. *Middle panel:* Changes in highest completed ratio; *right-hand panel:* Changes in break point*;* conventions as in left-hand panel.

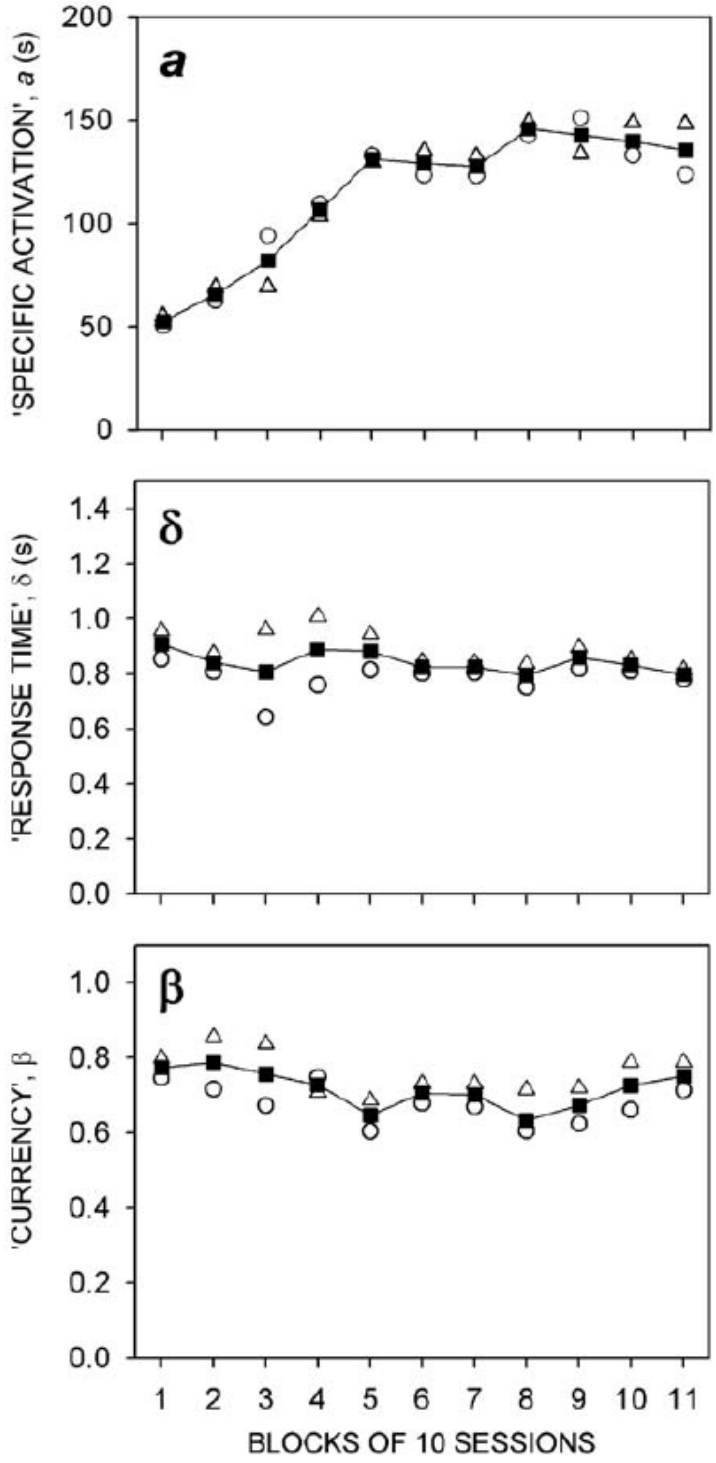

Figure 3. Values of the three parameters of Equation 1 (uper graph: *a*; middle graph: δ; lower graph: β) in successive blocks of 10 sessions during the pre-surgical training phase of the experiment. The filled symbols show the mean values for all rats; the open symbols show the mean values for the rats that were subsequently allocated to the sham-lesioned group (triangles) and the Ox-Sap lesioned group (circles).

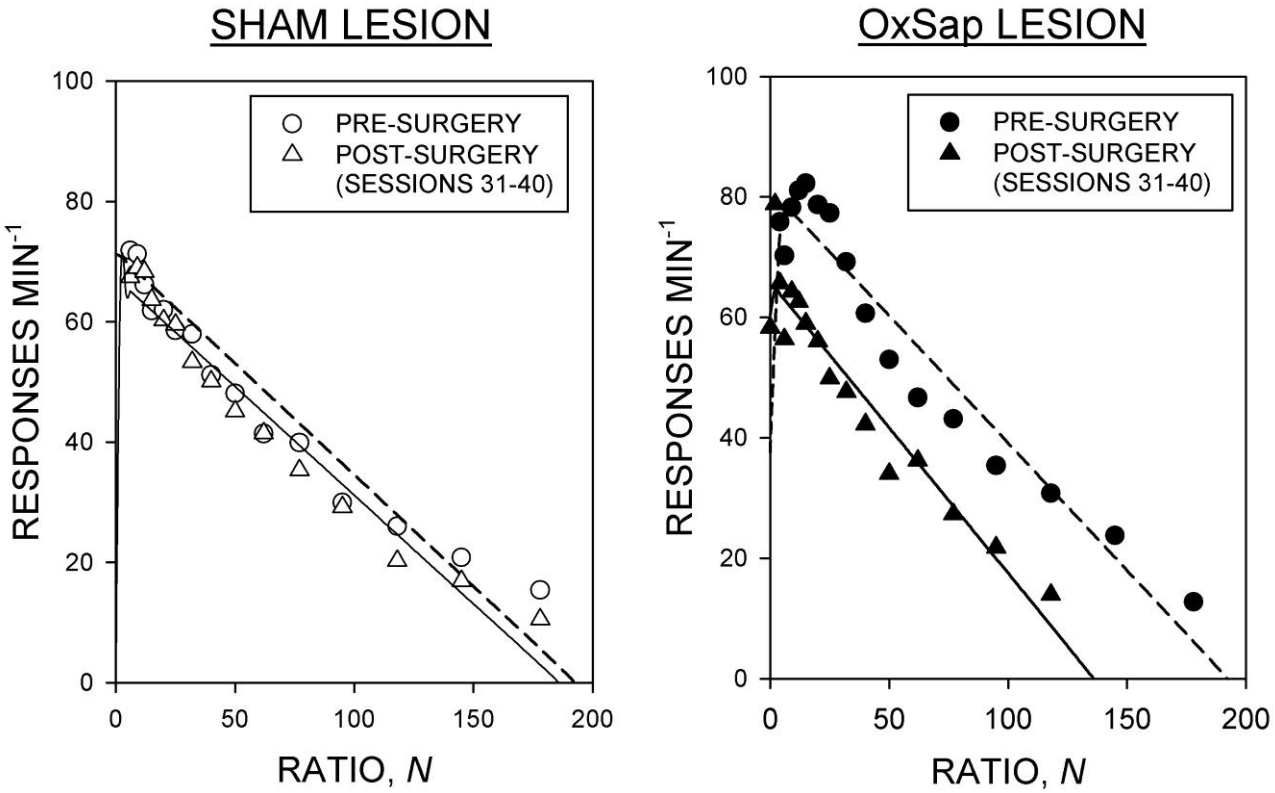

Figure 4. Overall response rates in successive ratios of the progressive-ratio schedule. *Left hand panel:* data from the sham-lesioned group; *right hand panel:* data from the OxSap-lesioned group. *Ordinates:* response rate (responses min^{-1}); *abscisae:* ratio, *N*. Points are group mean data. *Circles*: data from the last ten sessions before surgery; *triangles*: data from sessions 31-40 after surgery. The curves are fits of Equation 1 to the data (see text for details). Note the reduction of response rates in the OxSap-lesioned group, but not the sham-lesioned group, following surgery.

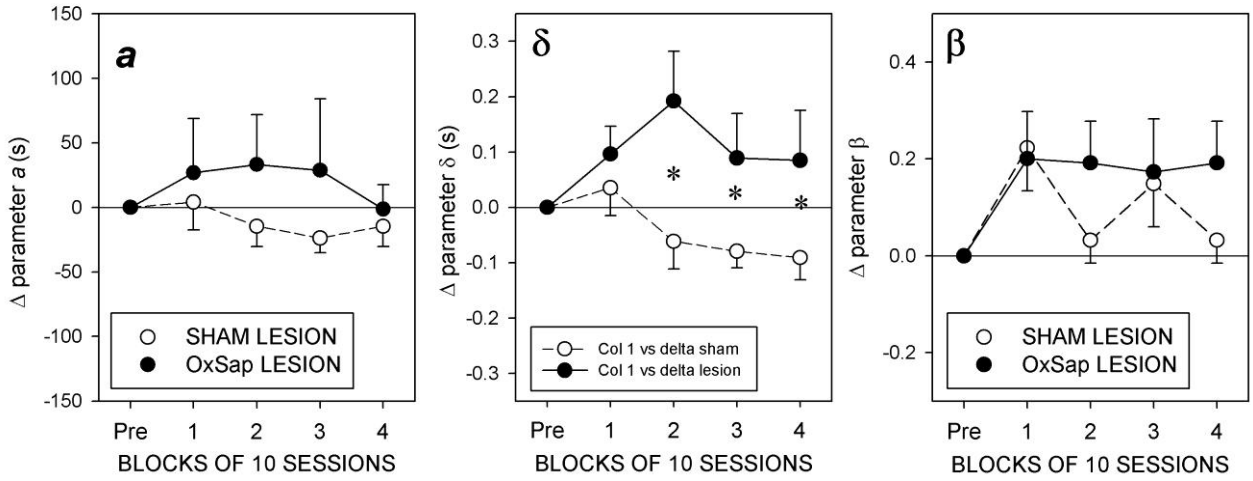

Figure 5. Changes in the parameters of Equation 1 following surgery in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups. *Ordinates*: change in parameter value; *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p<0.05$. *Left-hand graph*: 'activation' parameter, *a* (s); *middle graph*: 'response time' parameter, δ (s); *right-hand graph*: 'currency' parameter, β.

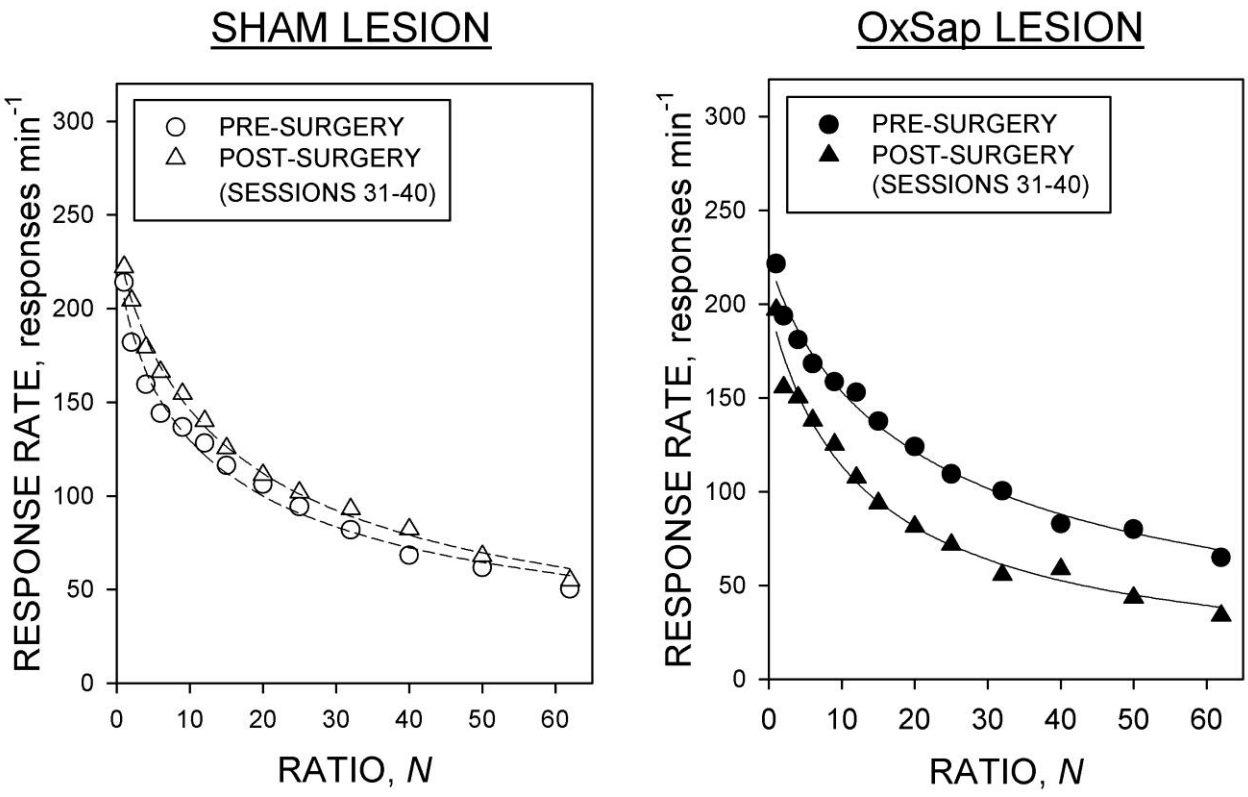

Figure 6. Running response rates in successive ratios of the progressive ratio schedule. *Left hand panel:* data from the sham-lesioned group; *right hand panel:* data from the OxSap-lesioned group. *Ordinates:* response rate (responses min^{-1}); *abscisae:* ratio, *N*. Points are group mean data . *Circles*: data from the last ten sessions before surgery; *triangles*: data from sessions 31-40 after surgery. Note the reduction of response rates in the OxSap-lesioned group, but not the sham-lesioned group, following surgery.

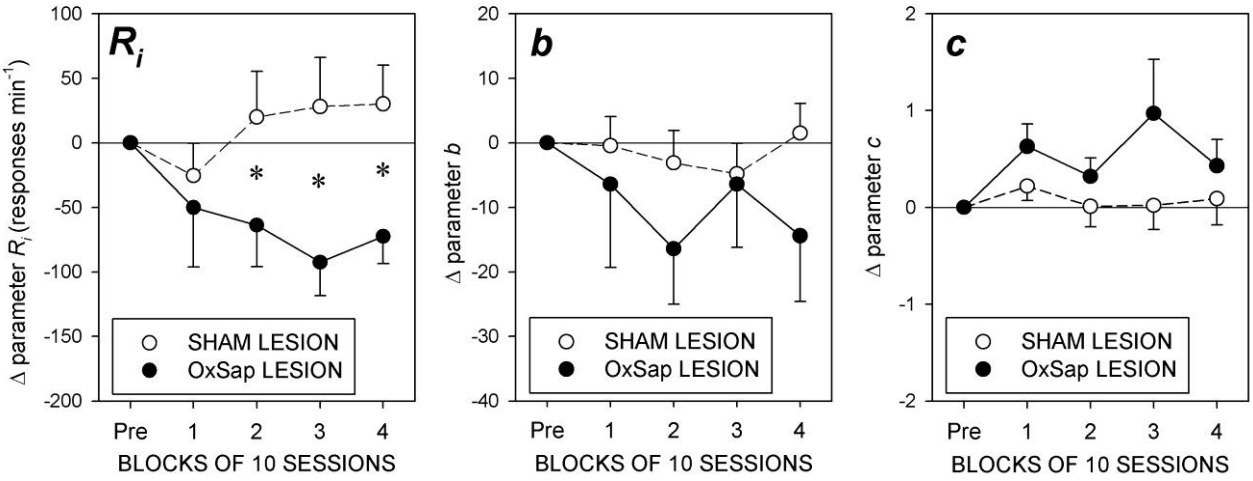

Figure 7. Changes in the parameters of Equation 2 following surgery in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups. *Ordinates*: change in parameter value; *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p<0.05$. *Left-hand graph*: 'initial response rate' parameter, R_i (responses min^{-1}); *middle graph*: 'decay' parameter, *b* (s); *right-hand graph*: 'exponent', *c*.

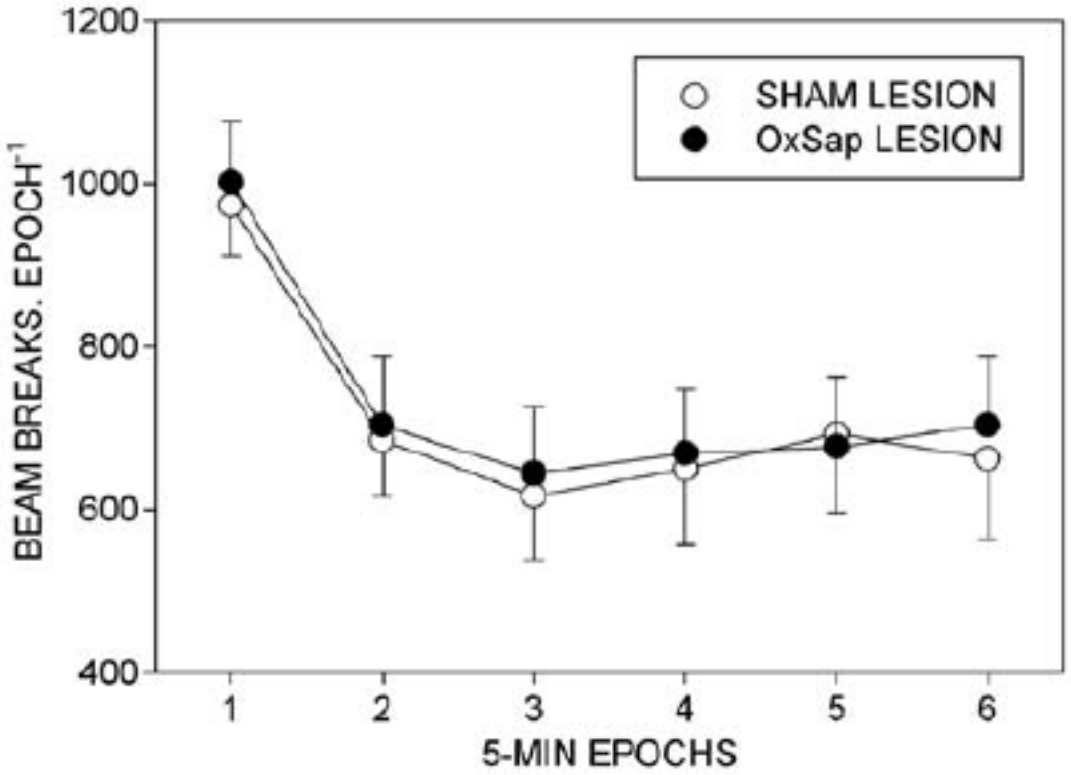

Figure 8. Locomotor activity in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups in 30-minute sessions. *Ordinate*: total beam breaks per 5-min epoch; *abscissa*: epochs. Points are group mean data (± SEM).

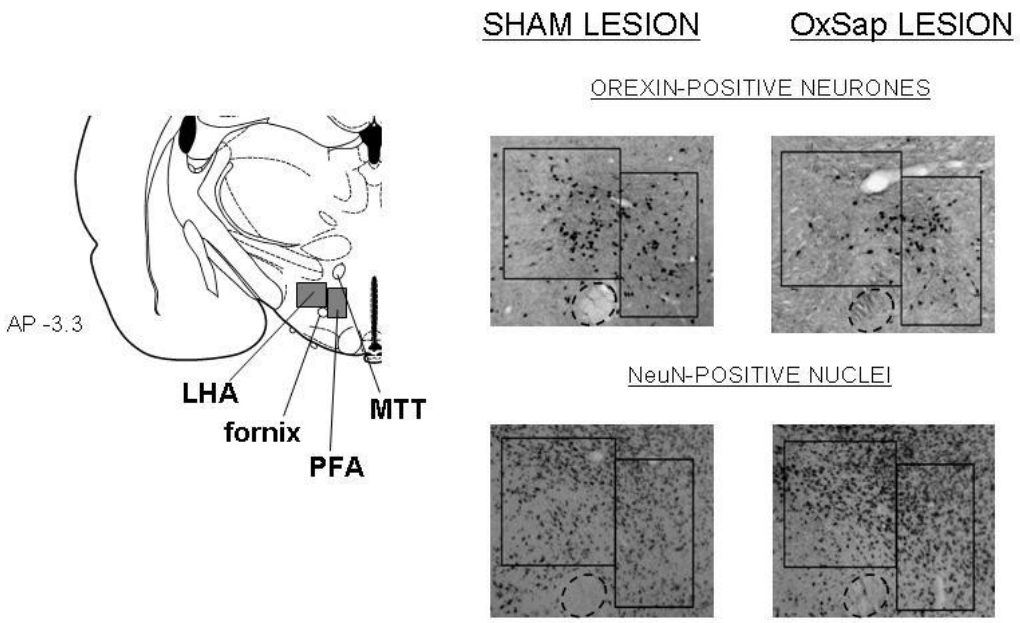

Figure 9. *Left-hand panel*. Diagram of coronal section of the rat brain at AP -3.3 mm, measured from bregma (from Paxinos and Watson 1998). The filled rectangles indicate the areas used for counting orexin-positive neurones and NeuN-positive nuclei. LHA, lateral hypothalamic area; PFA, perifornical hypothalamic area; MTT, mammillothalamic tract. Representative coronal sections through the hypothalamus of a sham-lesioned and an OxSap-lesioned rat are shown in the middle and right-hand panels; upper panels: sections stained for orexin; lower panels: sections stained for NeuN.

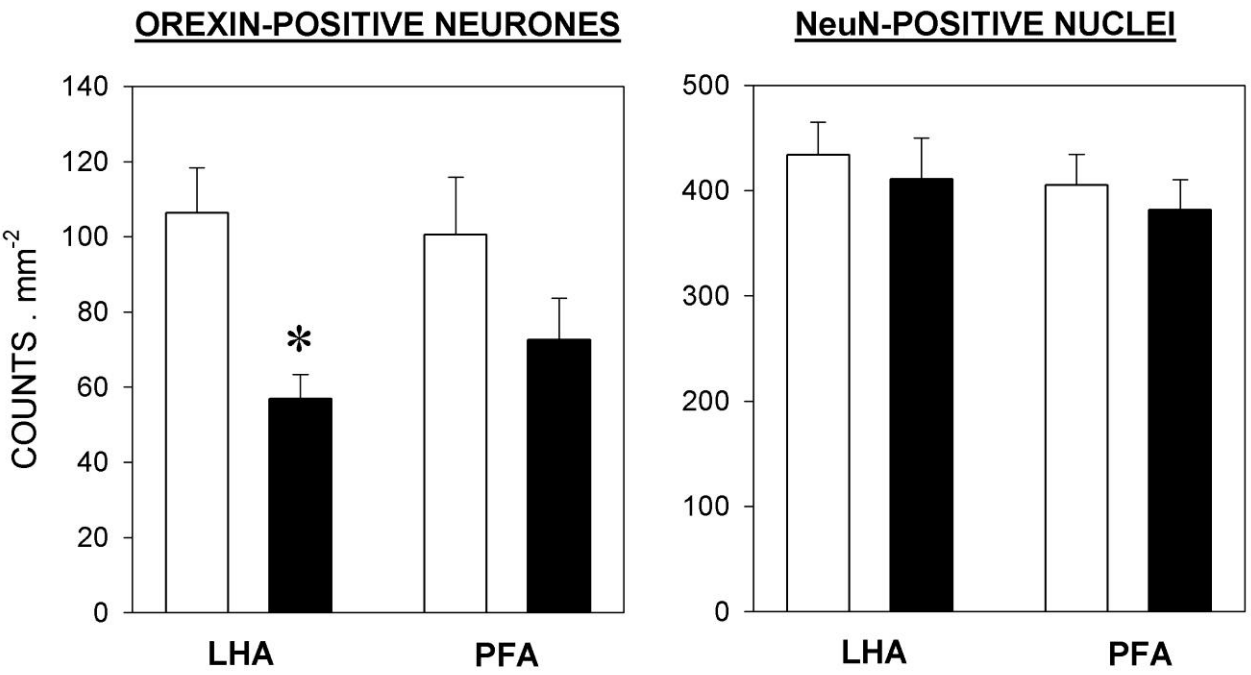

Figure 10. *Left-hand panel.* Density of orexin-positive neurones counted in the lateral hypothalamic area (LHA) and perifornical hypothalamic area (PFA). Ordinate: counts mm^{-2}. Columns show the group mean data (+SEM) for the sham-lesioned (white columns) and the OxSap-lesioned (black columns) rats. Difference between groups: * $p<0.05$. *Right-hand panel.* Density of NeuN-positive nuclei in the LHA and PFA of the two groups (conventions as in the left-hand panel).