European Journal of Orthodontics 24 (2002) 107–117

Emotional stress and brux-like activity of the masseter muscle in rats

Vincent Paul Rosales*, Koichi Ikeda*, Ko Hizaki**, Tetsurou Naruo**, Shin-ichi Nozoe** and Gakuji Ito*

*Department of Orthodontics, Kagoshima University Dental School, and **Department of Psychosomatic Medicine, Kagoshima University Hospital, Japan

SUMMARY The aim of this study was to further clarify the relationship between emotional stress and bruxism. In experiment 1, 60 male 9-week-old Wistar rats were divided into four groups: the emotionally stressed (ES), the emotionally non-stressed (NS), the electrically foot-shocked (FSd), and the non-foot-shocked (NSd). ES rats were confined in a communication box for one hour a day to observe the emotional responses of neighbouring FSd rats. On days 0, 1, 4, 8, and 12, the electromyographic activity of the ES and NS rats' left masseter muscles was recorded for one hour, three hours after confinement in the communication box. Brux-like activity appeared in the masseter muscle of the ES group on days 1, 4, 8, and 12, but not in the NS group.

In experiment 2, 36 male Wistar rats, 9 weeks old, were divided into three groups: emotionally stressed rats treated with an anti-anxiety drug (DES), emotionally stressed rats treated with saline as a vehicle (VES), and 24 FSd rats. Stress and EMG procedures were the same as those in experiment 1. Brux-like episodes decreased in DES rats from day 1 and significant differences were found on days 4 ($P < 0.01$), 8 ($P < 0.05$), and 12 ($P < 0.05$), when compared with the VES group. These findings suggest that emotional stress induces brux-like activity in the masseter muscle of rats, which was reduced with anti-anxiety drugs.

Introduction

Bruxism is widely accepted as a parafunctional oral habit characterized by non-functional clenching and grinding of teeth (Ramfjord, 1961), which results in excessive tooth wear, periodontal problems (Moore, 1956), and temporomandibular joint disorders (TMD; Trenouth, 1979; Rugh and Harlan, 1988). In the aetiology of bruxism, occlusal interference (Ramfjord and Ash, 1971; Dawson, 1974), and emotional and/or psychological stress (Rugh and Solberg, 1976; Glaros and Rao, 1977), have been highlighted as the most notable factors. However, the relevance of occlusal interference to bruxism remains questionable (Rugh *et al*., 1984; Okeson, 1995), while the connection between emotional stress and bruxism has received more attention (Klineberg, 1994).

Previous research suggests that emotional and/or psychological stress are associated with bruxism in humans as indicated by the increase of jaw muscle electromyographic (EMG) activity or combined with the increase of catecholamine levels (Rao and Glaros, 1979; Clark *et al*., 1980; Rugh and Harlan, 1988; Vanderas *et al*., 1999). Increase in jaw muscle activity was also observed on animal models that exhibited emotional states associated with bruxism (Landgren and Olsson, 1980; Weiner *et al*., 1993; Byrd, 1997; Sitthisomwong *et al*., 2000). However, these animal models required hypothalamic stimulation to detect their emotional responses when there was an increase of jaw muscle activities linked to bruxism (Landgren and Olsson, 1980; Weiner *et al*., 1993; Sitthisomwong *et al*., 2000). Although previous research associated emotional states with bruxism, there seems to be no thorough understanding of how emotional stress provokes masticatory parafunction, such as bruxism. Using animal models conditioned to an emotionally

stressful environment would further clarify the association of emotional stress to bruxism for a certain time period.

The present study was designed to induce experimental anxiety in rats using an emotional stress paradigm, employed by the intra-species emotional communication within a communication box (Ogawa *et al*., 1990). The hypothesis of this study was that emotional stress induces hyperactivity of masticatory muscles, which then results in oral parafunction such as bruxism.

Two experiments were carried out: experiment 1 determined whether exposure to emotional stress induced brux-like activity of the masseter muscles in rats, and experiment 2 verified whether blocking with an anti-anxiety drug would lessen the brux-like activity. The relevance of this study lies in the determination that there is a positive link between emotional and/or psychological stress and bruxism, which therefore supports a body of research demonstrating the important role of emotional and/or psychological stress in human bruxism.

Experiment 1: detection of brux-like activity induced by emotional stress

Animals and methods

Animals. Sixty male 9-week-old Wistar rats were divided into four groups: 10 emotionally stressed (ES), 10 emotionally non-stressed (NS), 20 electric foot-shocked (FSd), and 20 non-foot-shocked (NSd) rats. They were housed in a temperature-controlled room at 24°C under a 12-hour light: dark cycle, and given free access to food and water. Prior to the experiment, all rats were carefully habituated and conditioned to adapt themselves to the communication box for one hour a day for five days. Body weight was recorded daily for both ES and NS rats. The experimental procedures were reviewed and approved by the Ethics Committee of Kagoshima University Dental School.

Electrode installation. ES and NS rats were given pentobarbital anaesthesia (30 mg/kg) intraperitoneally. Five to ten minutes later, ketamine anaesthesia (15 mg/kg) was administered intramuscularly. The dorsal surface of the head was incised to expose the parietal bones, where three stainless steel screws (1 mm in diameter and 3 mm in length) were partially embedded on the parietal bones as stabilizing posts for a female miniature connector. Another incision was made on the left cheek to expose the left masseter muscle, where paired electrodes of polyurethane-coated copper wires (0.08 mm in diameter; Unique Medical Co., Tokyo, Japan) with bared tips of 1 mm in length were inserted at 3 mm inter-polar distance, using a 27-gauge hypodermic needle. Electrodes were sutured to the connective tissue to prevent displacement. The ends of the electrodes passed through the subcutaneous tunnel, emerged at the surface of parietal bones, were welded to two poles of the female miniature connector, and covered by silicon for insulation. The female miniature connector was stabilized with the ground electrode screw (3 mm in diameter and 10 mm in length) at the parietal bones with the acrylic resin. After surgery, an antibiotic (cefazolin sodium) was administered intramuscularly to prevent post-surgical infection.

Stress stimulation within the communication box. The communication box (Ogawa *et al*., 1990) was selected as the emotional stress paradigm in this study. It consists of 16 compartments (16×16 cm), separated by transparent plastic boards. The boards prevent each animal from physical contact, but allow them to receive cues such as visual, auditory, and olfactory sensations from the neighbouring animals. Each compartment was equipped with a grid floor of stainless steel rods, 5 mm in diameter, placed 1.3 cm apart. An electric generator (MATYS, Toyo Sangyo Co. Ltd, Toyama, Japan) was connected to the grid floors to produce an electric current of 2 mA to generate an electric foot shock for 10 seconds with an interval of 120 seconds. The grid floors of eight compartments were covered by plastic plates to prevent electric foot shock and served as non-foot-shock compartments for the ES rats (Figure 1).

Stress stimulation within the communication box commenced five days after electrode installation. Prior to the day of stress stimulation (day 0), ES rats together with FSd rats were

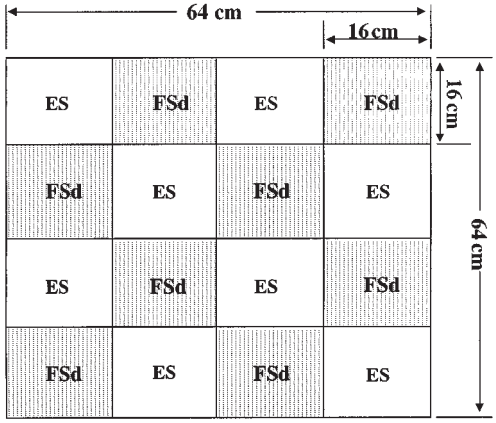

Figure 1 Arrangement of non-foot-shock (open areas) and foot-shock (shaded areas) compartments for ES and FSd rats in the communication box.

individually confined in each compartment of the communication box for one hour without any electric foot shock. From days 1 to 12, the electric foot shock was introduced to FSd rats (stress senders) for one hour a day at a fixed time in the morning. ES rats (stress responders) confined in the non-foot-shock compartments were then exposed to emotional cues from neighbouring FSd rats, such as shrieks, smells of urine or faeces, and jumping response. Consequently, ES rats were assumed to be in fear or in a state of anxiety (Ogawa *et al*., 1990).

Sham experiments were carried out on different days. NSd rats (stress senders) were placed in the foot-shock compartments and NS rats (stress responders) in the non-foot-shock compartments, but no electric foot shock was administered to NSd rats.

FSd and NSd groups were only used to send effective cues during stress and non-stress sessions, respectively.

Electromyography (EMG) recording. EMG activity of the left masseter muscle was recorded in both ES and NS groups on days 0, 1, 4, 8, and 12. Day 0 served as the baseline to detect any changes in muscle activity from days 1 to 12 of the experiment. Three hours after release from the communication box, all the ES and NS rats were placed in a transparent cage in an electronically shielded room. A leading cable of the male miniature connector was attached to the female miniature connector on the rat's head. The rat was allowed to move freely in the cage for 15 minutes before the recording and for one hour during the EMG recording.

EMG output was amplified and filtered (0.03–1 kHz) by AC amplifiers (Nihon Kohden Co., Tokyo, Japan), displayed on a dual-beam oscilloscope and stored using a data recorder (A-69, Sony Magnescale Inc., Tokyo, Japan) at a tape speed of 3.8 cm/seconds. During EMG recording, episodes of brux-like activity of the masseter muscle were established by both observing the jaw movement of the rat and listening to the sound of muscle activity on an audio monitor (Pohto, 1979; Byrd, 1997).

Total body, thymus, spleen, and adrenal gland weight. Body weight was examined on days 0, 1, 4, 8, and 12. On the last day of experiment, ES and NS rats were killed by ether anaesthesia immediately after EMG recording. Thymus, spleen, and adrenal glands were removed and weighed, since these organs are said to be affected by emotional stress (Marsh and Rasmussen, 1960).

Analysis of EMG data. EMG data were printed out for analysis of episodes of brux-like activity. The onset of brux-like activity was identified as an increase in the mean amplitude + 2 SD of the amplitude of the rest activity. The number and duration of episodes of brux-like activity within a one hour period were counted and measured. A Mann–Whitney *U*-test was used to compare the episodes of brux-like activity between ES and NS groups, and Wilcoxon's signed rank sum test was used to analyse the intra-group difference of the episodes of brux-like activity.

Results

Examples of raw EMG pattern of the masseter muscle in ES and NS rats are shown in Figure 2. The level of masseter muscle activity at rest was identified as 0.0585 mV. The number, duration, and total duration of episodes of brux-like activity of the masseter muscle within a one hour period in the ES group are shown in Table 1. On day 0, EMG activity of the masseter muscle was low, and did not differ between ES and NS rats.

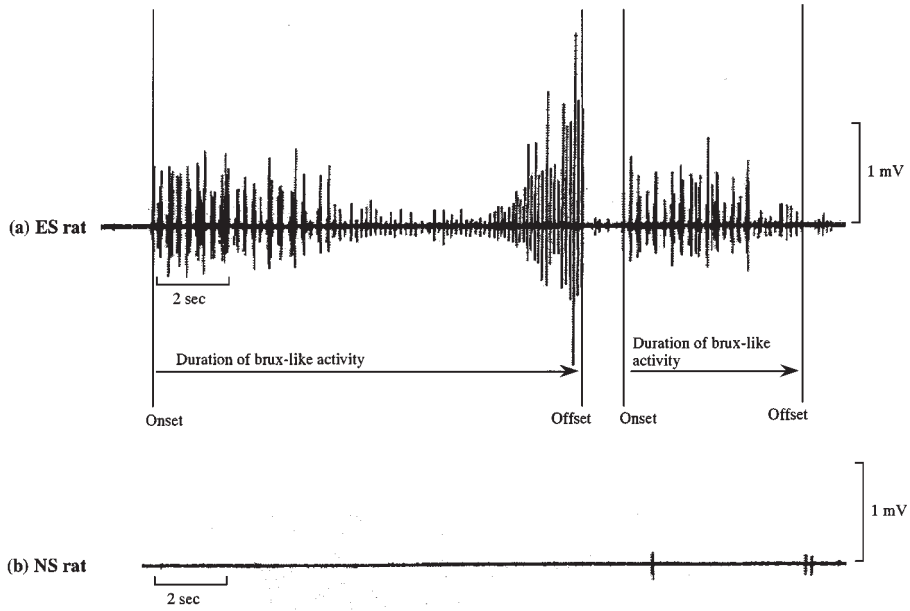

Figure 2 Examples of raw EMG patterns of the masseter muscle in ES and NS rats. (a) Brux-like activity in ES rats detected by the presence of cyclic bursts of large amplitude and longer duration. (b) NS rats showed only low-level EMG activity completely different from brux-like activity.

Table 1 Episodes of brux-like activity at the masseter muscle within a one hour period in the ES group.

	Day 0	Day 1	Day 4	Day 8	Day 12
Number of episodes	0	1.5 ± 1.1	1.9 ± 0.7	2.2 ± 0.8	1.5 ± 0.7
Total duration of episodes (seconds)	0	106.5 ± 71.6	133.2 ± 50.9	140.7 ± 43.7	99.7 ± 52.3
Mean duration of episodes (seconds)	0	67.1 ± 52.7	76.6 ± 29.9	79.1 ± 48.4	72.5 ± 46.0

Values indicate mean ± SEM. No episode of brux-like activity was found in the NS group.

From day 1, however, episodes of brux-like activity of the masseter muscle were detected by the presence of cyclic bursts of large amplitude and longer duration, which were continuously observed until day 12 in ES rats. The NS group, on the other hand, did not exhibit such activities throughout the experiment.

Episodes of brux-like activity on days 0, 1, 4, 8, and 12 in the ES and NS groups were compared (Figure 3). Basal EMG recording on day 0 was low in both groups. When stress stimulation began on day 1, episodes of brux-like activity in the ES group were significantly higher ($P < 0.001$) compared with the NS group. These episodes remained high on days 4 ($P < 0.001$), 8 ($P < 0.001$), and 12 ($P < 0.001$). The Wilcoxon's test for intra-group difference showed that episodes of brux-like activity in the ES group were higher on days 1 ($P < 0.05$), 4 ($P < 0.01$), 8 ($P < 0.01$), and 12 ($P < 0.01$) compared with day 0. There were no significant differences during days 1, 4, 8, and 12. No significant differences existed in the NS group from day 0 until day 12.

Body weight gain showed essentially no difference between either group throughout the experiment (Figure 4). There was no significant difference in the weight of thymus, spleen, and adrenals between the ES and NS groups on the last day of the experimental period (Figure 5).

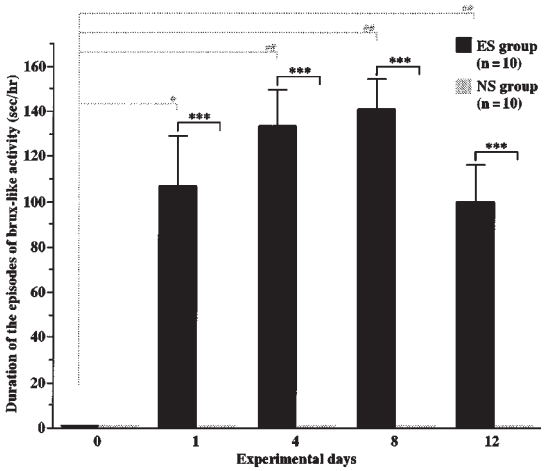

Figure 3 Duration of episodes of brux-like activity (mean ± SEM) in the ES and NS groups. ***$P < 0.001$ by Mann–Whitney *U*-test between the ES and NS groups. #$P < 0.05$; ##$P < 0.01$ by Wilcoxon's test for intra-group difference.

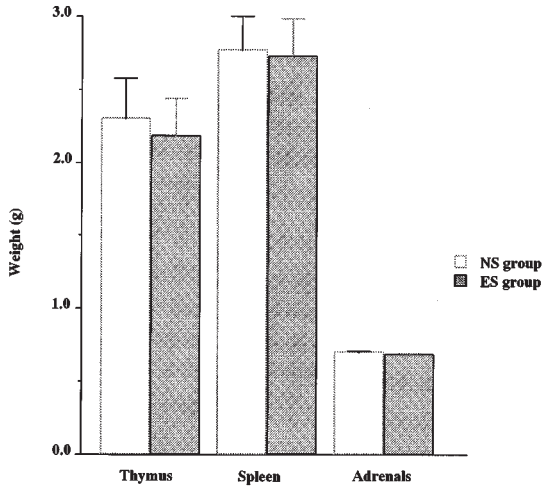

Figure 5 Weight of thymus, spleen, and adrenals (mean ± SD) at the end of the experimental period. No significant difference was found between the ES and NS groups.

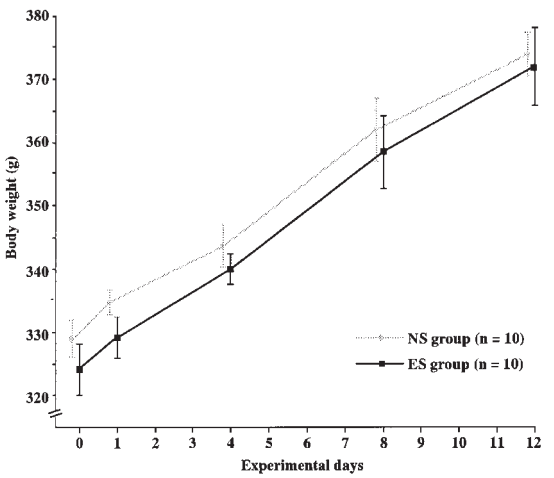

Figure 4 Changes in body weight (mean ± SD) during the experimental period in the ES and NS groups. No significant difference was found between either group.

Experiment 2: blocking of brux-like activity by an anti-anxiety drug

Animals and methods

Animals. Thirty-six male 9-week-old Wistar rats were divided into three groups: six emotionally stressed rats treated with the anti-anxiety drug (DES), six emotionally stressed rats injected with saline serving as the vehicle (VES), and 24 electric foot-shocked (FSd) rats. Habituation and conditioning procedures to ensure the rats' adaptation to the communication box were the same as those in experiment 1.

Administration of anti-anxiety drug. Diazepam has been suggested to possess inhibitory effects on the activation of mesoprefrontal dopamine neurons and hypothalamic-pituitary-adrenocortical axis induced by emotional and/or psychological stress (Kaneyuki *et al*., 1991). It has been used as an anti-anxiety drug in several stress-related experiments using the communication box (Ida *et al*., 1989; Kaneyuki *et al*., 1991; Ogawa *et al*., 1993). In order to verify the relationship of emotional stress and brux-like activity of the masseter muscle in ES rats as manifested in experiment 1, diazepam was chosen to block the effect of emotional stress on the episodes of brux-like activity.

Diazepam (Takeda Pharmacuetical Co., Osaka, Japan) was dissolved in saline containing 40 per cent propylene glycol. From days 1 to 12, diazepam (1 mg/kg) was injected subcutaneously in DES rats 30 minutes before

stress stimulation. For the sham experiment, saline was injected subcutaneously in VES rats.

Stress stimulation in the communication box. Using the same stress stimulation, including the same communication box as experiment 1, DES and VES rats (stress responders) received the stress responses from FSd rats (stress senders).

Prior to the day of stress stimulation (day 0), DES and FSd rats were confined in the communication box for one hour without administration of diazepam or electric foot shock. From days 1 to 12, DES rats administered with diazepam and FSd rats were separately confined inside the communication box, followed by the electric foot shock to FSd rats for one hour. DES rats readily perceived the responses of FSd rats, such as shrieks, jumping, and smells of urine or faeces. VES rats with their corresponding FSd rats underwent the same procedure as the DES rats.

Recording and analysis of EMG data. EMG recording of the left masseter muscle on days 0, 1, 4, 8, and 12 in DES and VES groups, and analysis of their EMG data were exactly the same as those in experiment 1.

Results

Episodes of brux-like activity on days 0, 1, 4, 8, and 12 in the DES and VES groups are shown in Figure 6. Basal EMG recordings on day 0 were low in both groups. When the stress stimulation began on day 1, episodes of brux-like activity appeared lower in the DES group compared with the VES group, but the difference was not significant. Episodes of brux-like activity in the DES group remained significantly low on days 4 ($P < 0.01$), 8 ($P < 0.05$), and 12 ($P < 0.05$), when compared with the VES group. Episodes of brux-like activity in the DES group were minimal from day 1, which eventually decreased until day 12 without any significant differences (Wilcoxon's test of intra-group difference). However, episodes of brux-like activity in the VES group were significantly higher on days 4 ($P < 0.05$) and 8 ($P < 0.05$) compared with day 0. There were no significant differences during days 1, 4, 8, and 12.

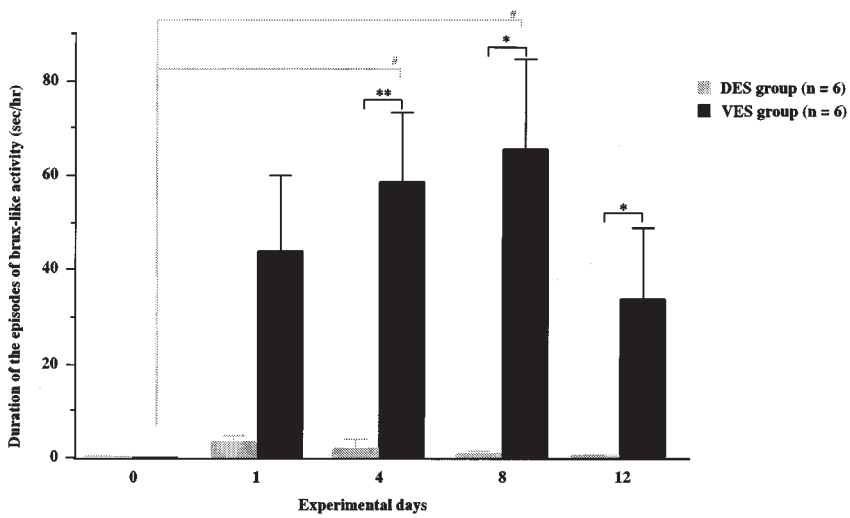

Figure 6 Duration of episodes of brux-like activity (mean ± SEM) in emotionally stressed rats treated with diazepam (DES) and emotionally stressed rats treated with saline as vehicle (VES). *$P < 0.05$, **$P < 0.01$ by Mann–Whitney *U*-test between DES and VES groups. #$P < 0.05$ by Wilcoxon's test for intra-group difference.

Discussion

Emotional stress and brux-like activity of masseter muscle

According to Ogawa *et al.* (1990), the communication box method can develop emotional stress in animals as they perceive the responses of other animals exposed to physical stress delivered through an electric foot shock. This kind of intra-species emotional communication has been detected in earlier studies, and further confirms that animals subjected to experimental anxiety within the communication box increase their stress hormone (plasma corticosterone level) and some develop stomach ulcers (Ogawa *et al.*, 1990; Ishikawa *et al.*, 1992). In the present study, the communication box was used to simulate an emotionally stressful environment and to determine whether oral parafunction, such as brux-like activity, would occur in the masseter muscles of rats exposed to emotional stress.

Experimental bruxism and/or masticatory muscle hyperactivity has been recorded in animals subjected to stressors (Pohto, 1979; Landgren and Olsson, 1980; Weiner *et al.*, 1993; Richardin *et al.*, 1995; Byrd, 1997; Sitthisomwong *et al.*, 2000). One oral sign of bruxism is an increase in masseter muscle activity in humans (Sherman, 1985) and in animals (Landgren and Olsson, 1980; Yamada *et al.*, 1990; Weiner *et al.*, 1993; Shoji *et al.*, 1994; Sitthisomwong *et al.*, 2000). In animal studies, EMG of the masseter muscle shows cyclic bursts with a longer duration and larger amplitude particularly in the grinding type of bruxism or non-functional masticatory activity (Yamada *et al.*, 1990; Shoji *et al.*, 1994). Such cyclic bursts of EMG in animals was termed as brux-like activity in the present study (Figure 2). The basal EMG level of masseter muscle activity was first determined under the normal or non-stressed condition on day 0. Both ES and NS groups showed a fairly low level of EMG activity. On day 1, EMG activity of the masseter muscle identified significant episodic cyclic bursts of brux-like activity after the first exposure to emotional stress in the ES group, while such activity was not evident in the NS group (Figure 3). The presence of brux-like activity in ES rats may indicate that emotional stress stimuli augmented the masseter muscle activity. On days 4, 8, and 12, episodes of brux-like activity in the ES group remained significantly high. The intra-group effects on days 1, 4, 8, and 12 in the ES rats were significantly different from day 0. This marked difference across the days demonstrated the effect of emotional stress in jaw muscle function. The brux-like episodes decreased with no significant difference from days 8 to 12 in the ES group. This may indicate that the ES group had learned to cope with the stressful environment.

In the present study, no episode of brux-like activity was observed from days 0 to 12 in the NS group, indicating that this group did not experience emotional stress during the entire experimental period. The ES group, however, had brux-like episodes from days 1 to 12 and this illustrates their emotionally stressful experience, as found in previous studies (Pohto, 1979; Byrd, 1997). Although the present investigation was limited to the brux-like activity of jaw muscles, these findings support the notion that changes in the emotional state are reflected by muscle tension (Sainsbury and Gibson, 1954) and, in particular, by tension of the masticatory muscles (Burstone, 1946; Perry *et al.*, 1960). Brux-like activity observed in anxious rats in the present investigation may suggest the role of a stress-related event to jaw muscle function, which leads to jaw movement disorders.

Changes in the weight of the thymus, spleen, and adrenal glands are usually found when stress is induced (Marsh and Rasmussen, 1960); however, Ishikawa *et al.* (1995) observed no change in the weight of such organs or body weight in rats exposed to emotional stress. The present results also showed no difference in the organ weights between the ES and NS groups on the last experimental day. Lack of weight reduction in these organs, particularly in the ES rats, may indicate that the effect of emotional stress induced was not sufficiently strong to produce a reduction of weight in these organs, but was sufficient to produce brux-like activity of the masseter muscle.

Blocking of stress-induced brux-like activity by the anti-anxiety drug

Stress provoking stimuli are known to activate the dopaminergic system (Bliss and Ailion, 1971;

Thiery *et al*., 1976). According to Ida *et al*. (1989), the mesoprefrontal dopamine system plays an important role in the control of negative states, such as fear and/or anxiety. Previous studies have demonstrated that the activation of the mesoprefrontal dopamine system is induced by emotional stress and reversed by the administration of diazepam (Ida *et al*., 1989; Kaneyuki *et al*., 1991). When the stress stimulation was commenced in the present study, episodes of brux-like activity appeared lower in the DES group on day 1 and remained significantly low on days 4, 8, and 12, compared with the VES group (Figure 6). Episodes of brux-like activity in the DES group were minimal from day 1 and decreased until day 12 without any significant differences. However, episodes of brux-like activity in VES group were significantly high on days 4 and 8 compared with day 0, as observed in the ES group in experiment 1. The decreasing bruxing episodes of the masseter muscle in DES group may indicate the gradual blocking effect of diazepam. The strong potency of diazepam was found to suppress the brux-like activity.

Pohto *et al*. (1979) demonstrated that diazepam does not prevent bruxism in rats when this is induced by a combination of apomorphine and occlusal interference. In the present investigation, however, the brux-like episodes markedly decreased when diazepam was used as a blocking agent in emotional stress-induced brux-like activity. This may indicate that the effect of diazepam depends on the aetiological factors of oral parafunction. Since the present study used only masseter EMG recordings to demonstrate the drug effect on emotional stress-induced brux-like activity, further research is being undertaken to determine the neural activity of emotional stress using the neuro-endocrinological method.

The present study attempts to link emotional states and masseter muscle activity, and the results support a pathophysiological mechanism and therapeutic implications. First, from the pathophysiological mechanism, emotional states, as in stress, anxiety, and startle responses, are rooted mainly on subcortical networks. Research has identified the amygdala of the limbic system as specifically concerned with emotions. The direct thalamic inputs of short latency mediate primitive emotional responses, and prepare the amygdala (the basolateral division) for the reception of more sophisticated information about cognitive representation of emotion from higher centres, such as the ventromedial prefrontal cortex. The output of amygdala, as well as afferent input that is triggered by activity of autonomic effectors, feeds back to cortical structures to give rise to conscious emotional experience. As a result, the projection from the thalamus to the amygdala may allow primitive sensory representation rapidly to activate amygdala, an activation that may be important in situations of stress and danger. The efferents from the amygdala (mainly coming from the central nucleus) send two pathways and these are: (1) the stria terminalis that innervates the hypothalamus, bed nucleus of the stria terminalis and nucleus accumbens; and (2) the ventral amygdalofugal pathway that provides input to the brainstem, dorsomedial nucleus of thalamus and rostral cingulate gyrus. Based on the Davis model of emotions, both conditioned and unconditioned fear stimuli activate the amygdala, which in turn send output signals to the different brain structures to include autonomic centres, respiratory centres, and brainstem reticular formation, including the trigeminal and facial motor nuclei. The effects on cranial nerve nuclei and motor components (cranial nerve V, masseters; cranial nerve VII, muscles of facial expression) lead to mouth opening and jaw movements that are supposedly facial expressions of fear (Davis, 1992; Kandel *et al*., 1995). Secondly, from the therapeutic implications, benzodiazepines (e.g. diazepam as used in the present experiment) are pharmacologically linked to GABAergic (gamma aminobutyric acid) neurotransmission. There are two known subtypes of GABA receptors, namely GABA-A and GABA-B. GABA-A receptors are allosterically linked to benzodiazepine receptor sites (i.e. anxiolytics such as diazepam), including non-benzodiazepine receptor sites (i.e. for sedative-hypnotics such as zolpidem and even alcohol). The GABA-B receptors are non-allosterically linked to benzodiazepines, but bind selectively to the muscle relaxant-anti-spasticity drug baclofen. The receptor complex is hypothetically responsible in part for mediating such wide-ranging central nervous system activities as seizures, anti-convulsant drug

effects, and the behavioural effects of alcohol, as well as the known anxiolytic, sedative-hypnotic, and muscle relaxant effects of the benzodiazepines. Depending on the receptor subtype, the benzodiazepine receptors are located in several areas of the nervous system (e.g. cerebellum and brainstem, spinal cord, and striatum) and peripherally (e.g. kidneys). The pathomechanism underlies the fact that GABA-A receptors interact with the chloride channel to open it. Once the chloride channel is open, the action is inhibitory to that neuron and the reason why GABA is largely known as an inhibitory neurotransmitter. On administering benzodiazepine drugs, the benzodiazepine receptors are activated and, since they are allosterically linked to GABA-A receptors, they modulate the chloride channel opening indirectly and induce inhibition of that neuronal function. Modulation of the GABA–benzodiazepine receptor complex is therefore not only thought to underlie the pharmacological actions of anti-anxiety drugs, but is also theorized to serve as the vehicle for mediating the emotion of anxiety itself. It has been speculated, for example, that reduced actions of GABA and the postulated endogenous benzodiazepines at this receptor complex may be associated with the emotion of anxiety, whether the emotion is normal or pathological (Stahl, 2000). From the clinical level, the fact that panic symptoms respond to benzodiazepine medication and that infusion of benzodiazepine antagonists causes an intense terror-like experience in animals and in humans provides support for a potential role for abnormalities of this system in patients suffering from panic (Dorow *et al*., 1983; Shear, 1997).

Earlier animal studies (Pohto, 1979; Budz-Jorgensen, 1980; Byrd, 1997) support the assumption that emotional and/or psychological stress factors play an important part in human bruxism (Rao and Glaros, 1979; Clark *et al*., 1980; Rugh and Harlan, 1988; Vanderas *et al*., 1999). The findings of the present investigation support the related assumption that emotional and/or psychological stress contributes to bruxism, and its validity is based on the occurrence of brux-like activity in rats after repeated emotional stress in the communication box. Although brux-like episodes were evident in this investigation after exposure to emotional stress, the mechanism that triggers this oral parafunction is not yet fully understood. It seems that the dopaminergic system has a special role in oral parafunction that may be precipitated by emotional stress. Further studies are needed to confirm the influence of emotional stress on the dopaminergic system and its relevance to bruxism.

Two important insights can, however, be derived from this present experiment. Emotional stressors induce masseter muscle contractions that may be based on a pathway and may, in part, be mediated as follows: sensory inputs occur via the thalamus, with mainly visual and auditory cues from foot-shocked rats (activation of the amygdala, especially basolateral complex). Efferents to various CNS systems (e.g. hypothalamus and reticular formation), including muscular contraction relevant to the present experiment, via ventral amygdalofugal pathway to the brainstem, specifically the trigeminal motor neurons. Benzodiazepines alleviate this emotional stress by way of their action on the benzodiazepine–GABA-A receptor complex and modulate the opening of chloride channels. This level of action may in part be responsible for the anxiolytic effects or muscle relaxation that lead to reduction in masseter muscle contraction in the present experiment. These insights lend credence to the clinical state of anxiety and response to benzodiazepines. Since the present experiment was not designed to investigate other stress-related changes, the level of muscular and brux-like activities can only be speculated.

Clinically, the present results emphasize the importance of environmental influences on the emotional state that could affect the oral function. Difficulty in coping with life stress by certain individuals may predispose to TMD triggered by bruxism.

Address for correspondence

Professor Gakuji Ito
Department of Orthodontics
Kagoshima University Dental School
8-35-1 Sakuragaoka
Kagoshima 890-8544, Japan

Acknowledgements

The authors wish to express their sincere thanks to Professor Katsushi Yamada, Department of Hospital Pharmacy, Kagoshima University, Dr Masahiro Ishikawa, Meiji Seika Kaisha, Tokyo, Japan, and Dr Raymond Rosales, Division of Neurology and Psychiatry, UST Hospital, Manila, Philippines, for their valuable advice on this experiment, to Ms Junko Tsutsui, Psychosomatic Medicine, Kagoshima University, for the statistical methods, and Ms Suzette Rosales Cody for the English translation.

References

Bliss E L, Ailion J 1971 Relationship of stress and activity to brain dopamine and homovanillic acid. Life Sciences 10: 1161–1169

Budtz-Jorgensen E 1980 A 3-month study in monkeys of occlusal dysfunction and stress. Scandinavian Journal of Dental Research 88: 171–180

Burstone M S 1946 The psychosomatic aspects of dental problems. Journal of the American Dental Association 33: 862–871

Byrd K E 1997 Characterization of brux-like movements in the laboratory rat by optoelectronic mandibular tracking and electromyographic techniques. Archives of Oral Biology 42: 33–43

Clark G T, Rugh J D, Handelman S L 1980 Nocturnal masseter muscle activity and urinary catecholamine levels in bruxers. Journal of Dental Research 59: 1571–1576

Davis M 1992 The role of amygdala in fear and anxiety. Annual Review Neuroscience 15: 353–375

Dawson P E 1974 Evaluation, diagnosis and treatment of occlusal problems. C V Mosby, St Louis

Dorow R, Horowski R, Paschelke G 1983 Severe anxiety induced by FG7142, a b-carboline ligand for benzodiazepine receptors. Lancet 2: 98–99

Glaros A G, Rao S M 1977 Bruxism: a critical review. Psychological Bulletin 84: 767–781

Ida Y, Tsuda A, Sueyoshi K, Shirao I, Tanaka M 1989 Blockage of diazepam of conditioned fear-induced activation of rat mesoprefrontal dopamine neurons. Pharmacology, Biochemistry and Behavior 33: 477–479

Ishikawa M, Hara C, Ohdo S, Ogawa N 1992 Plasma corticosterone response of rats with sociopsychological stress in the communication box. Physiology and Behavior 52: 475–480

Ishikawa M, Ohdo S, Watanabe H, Hara C, Ogawa N 1995 Alteration in circadian rhythm of plasma corticosterone in rats following sociopsychological stress induced by communication box. Physiology and Behavior 57: 41–47

Kandel E R, Schwartz J H, Jessell T M (eds) 1995 Emotional states. In: Essentials of neural science and behavior. Prentice Hall International Inc., pp. 595–612

Kaneyuki H, Yokoo H, Tsuda A, Yoshida M, Mizuki Y, Yamada M, Tanaka M 1991 Psychological stress increases dopamine turnover selectively in mesoprefrontal dopamine neurons of rats: reversal by diazepam. Brain Research 557: 154–161

Klineberg I 1994 Bruxism: aetiology, clinical signs and symptoms. Australian Prosthodontic Journal 8: 9–17

Landgren S, Olsson K A 1980 The effect of electrical stimulation in the hypothalamus on the monosynaptic jaw closing and the disynaptic jaw opening reflexes in the cat. Experimental Brain Research 39: 389–400

Marsh J T, Rasmussen A F Jr 1960 Response of adrenals, thymus, spleen, and leukocytes to shuttle box and confinement stress. Proceedings of the Society for Experimental Biology and Medicine 104: 180–183

Moore D S 1956 Bruxism, diagnosis and treatment. Journal of Periodontology 27: 277–283

Ogawa N, Hara C, Ishikawa M 1990 Characteristics of socio-psychological stress induced by the communication box method in mice and rats. In: Manninen O (ed.) Environmental stress. ACES Publishing Ltd, Tampele, pp. 417–427

Ogawa N, Hara C, Takaki S 1993 Anxiolytic activity of SC-48274 compared with those of buspirone and diazepam in experimental anxiety models. Japan Journal of Pharmacology 61: 115–121

Okeson J P 1995 Occlusion and functional disorders of the masticatory system. Dental Clinics of North America 39: 285–300

Perry H T, Lammie G A, Main J, Teuscher G W 1960 Occlusion in stress situation. Journal of the American Dental Association 60: 626–633

Pohto P 1979 Experimental aggression and bruxism in rats. Acta Odontologica Scandinavica 37: 117–126

Pohto P, Sunden-Kuronen B, Alanen E 1979 Effect of occlusal interference, sedation and centrally acting muscle relaxants on apomorphine-induced oral dyskenesia in rats. Proceedings of the Finnish Dental Society 75: 85–94

Ramfjord S P 1961 Bruxism, a clinical and electromyographic study. Journal of the American Dental Association 62: 21–44

Ramfjord S P, Ash M M 1971 Occlusion, 2nd edn. W B Saunders Co., Philadelphia

Rao S M, Glaros A G 1979 Electromyographic correlates of experimentally induced stress in diurnal bruxists and normals. Journal of Dental Research 58: 1872–1878

Richardin P, Westphal A, Divry M, Didier G 1995 Influence of stress and occlusal interference on the EMG activity of some masticatory muscles during a single mastication cycle. Journal of Oral Rehabilitation 22: 775–780

Rugh J D, Harlan J 1988 Nocturial bruxism and temporomandibular disorders. Advances in Neurology 49: 329–341

Rugh J D, Solberg W K 1976 Psychological implications in temporomandibular pain and dysfunction. Oral Science Review 7: 3–30

Rugh J D, Barghi N, Drago C J 1984 Experimental occlusal discrepancies and nocturnal bruxism. Journal of Prosthetic Dentistry 51: 548–553

Sainsbury P, Gibson J G 1954 Symptoms of anxiety and tension and the accompanying physiological changes in the muscular system. Journal of Neurology, Neurosurgery and Psychiatry 17: 216–224

Shear M K 1997 Panic disorder with and without agoraphobia. In: Tasman A, Kay J, Lieberman J A (eds) Psychiatry. W B Saunders Co., Philadelphia, pp. 1020–1036

Sherman R A 1985 Relationships between jaw pain and jaw muscle contraction level: underlying factors and treatment effectiveness. Journal of Prosthetic Dentistry 54: 114–118

Shoji Y N, Bruce I C, Sui L Y L 1994 Electromyographic assessment of non-functional masseter muscle in an awake animal model. Journal of Craniomandibular Practice 12: 110–113

Sitthisomwong P, Weiner S, Levin L, Reisman S, Siegel A 2000 The effect of a cholecystokinin agonist on masseter muscle activity in the cat. Journal of Dental Research 79: 1823–1828

Stahl S M (ed.) 2000 Anxiolytics and sedative hypnotics. In: Essential pharmacology, neuroscientific basis and practical applications, 2nd edn. Cambridge University Press, Cambridge, pp. 297–333

Thierry A M, Tassin J P, Blanc G, Glowinski J 1976 Selective activation of the mesocortical DA system by stress. Nature 263: 242–244

Trenouth M J 1979 The relationship between bruxism and temporomandibular joint dysfunction as shown by computer analysis of nocturnal tooth contact patterns. Journal of Oral Rehabilitation 6: 81–87

Vanderas A P, Menenakou M, Kouimtzis T H, Papagiannoulis L 1999 Urinary catecolamine levels and bruxism in children. Journal of Oral Rehabilitation 26: 103–110

Weiner S, Shaikh M B, Siegel A 1993 Electromyographic activity in the masseter muscle resulting from stimulation of hypothalamic behavioral sites in the cat. Journal of Orofacial Pain 7: 370–377

Yamada Y, Uchida K, Sato T 1990 Mandibular movement trajectories and masticatory muscle activities in the rabbit in the sleep and wake states. Dentistry in Japan 27: 35–39