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REVIEW

SEROTONERGIC AND BENZODIAZEPINE MODULATION OF AGONISTIC BEHAVIOUR: ETHOPHARMACOLOGICAL ANALYSES

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ABSTRACT

The present article summarizes our recent work on the contribution of serotonergic (5-HT) and benzodiazepine (BDZ) receptors in agonistic behaviour using ethopharmacological technology. After an introduction, in which the basic principles of ethopharmacology of agonistic behaviour in rats are explained, our work on serotonin and benzodiazepines is illustrated using four animal aggression paradigms, viz. resident-intruder, colony, hypothalamically-induced aggression (EBS) and maternal aggression in lactating female rats.

Using a broad scala of serotonergic receptor ligands, including drugs influencing 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2 and 5-HT_3 receptors (both agonistic and antagonistic), we were able to postulate that only the 5-HT_{1B} - receptor is specifically involved in the modulation of offensive aggressive behaviour in rats. Other receptor types are either not involved or in a nonspecific manner.

Based on such ethopharmacological techniques we have developed specific antiagressive drugs, serenics. These drugs

have a high affinity for the 5-HT_{1B} receptor, thereby confirming our hypothesis.

In contrast to serotonin agonists, BDZ-agonists (benzodiazepines) enhance aggressive behaviour, at least at low dosages. This socalled pro-aggressive action was subject to extensive ethological investigations and appeared to depend on baseline levels of aggression, type of opponent, level of experience and type of paradigm used.

Ethopharmacology is a very worthwhile approach when trying to develop specific drugs (e.g. serenics) or unravelling the (behavioural) mechanism of action and the underlying motivational aspects (anxiety, depression, etc).

KEY WORDS: Ethopharmacology - Agonistic behaviour - Serotonin - Benzodiazepines - Serenics.

RESUMO

O presente artigo sintetiza nossas recentes contribuições sobre os papéis dos receptores serotonérgicos (5-HT) e benzodiazepínicos (BDZ), usando a técnica etofarmacológica, no comportamento agonístico. Após uma introdução, na qual os princípios básicos da etofarmacologia do comportamento agonístico de ratos são explicados, nosso trabalho sobre serotonina e benzodiazepínicos é ilustrado através da utilização de quatro paradigmas de agressão animal: residente-intruso, colonia estabilizada, agressão hipotalamicamente induzida (EBS) e agressão materna, em ratas lactantes.

Utilizando uma ampla gama de ligantes de receptores serotonérgicos, incluindo drogas que influenciam os receptores 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2 e 5-HT_3 (tanto agonísticos quanto antagonísticos), foi possível concluir que somente o receptor 5-HT_{1B} está especificamente envolvido na modulação do comportamento agressivo ofensivo em ratos. Outros tipos de receptores não estão envolvidos de modo específico ou não-específico no comportamento agressivo.

Baseados em tais técnicas etofarmacológicas, nós desenvolvemos drogas antiagressivas, os serênicos. Essas drogas tem uma grande afinidade com o receptor 5-HT_{1B}, confirmando, deste modo, nossa hipótese.

Em contraste com agonistas de serotonina, os agonistas benzodiazepínicos aumentam o comportamento agressivo, mesmo em pequenas doses. A chamada ação pró-agressiva foi submetida a uma minuciosa investigação e parece depender de uma linha de base prévia de agressão, tipo de oponente, nível de experiência e tipo de paradigma utilizado.

A etofarmacologia é uma abordagem muito útil para o desenvolvimento de drogas específicas (e.g. serênicos), ou para desvendar seu mecanismo de ação (comportamento) e seus aspectos motivacionais subjacentes (ansiedade, depressão etc.).

MODULATION OF AGONISTIC BEHAVIOUR

UNITERMOS: Etofarmacologia - Comportamento Agonístico
Serotonina - Benzodiazepínicos - Serênicos.

Introduction

The last decades have shown an increasing interest in the ethology, ecology, pharmacology, physiology, genetics and endocrinology of agonistic behaviour in vertebrates (cf. Huntingford and Tuerner, 1987). Endeavor in all these and other disciplines has led to increasing knowledge about the neurophysiology, neuroanatomy and neuropharmacology of agonistic behaviour (cf. Brain and Benton, 1981a,b; Olivier **et al.**, 1987a). For practical reasons most of these studies have been performed on rodents and especially psycho-pharmacological studies have used rats and mice (Miczek, 1987), although primates contribute considerably to the emerging scientific knowledge of agonistic behaviour (Miczek, 1983).

Agonistic behaviour or animal conflict is a multidimensional and very complex phenomenon, especially in evolutionary higher species. Agonistic behaviour like all behaviour does not occur in a biological vacuum, but is dependent on all factors involved in a homeostatic regulatory system (cf. Archer, 1976; Wiepkema, 1987), modulated by internal and external signals. Important for the emergence of every aspect of agonistic behaviour are signals coming from the outside world, especially threatening stimuli like male rivals or predators. Depending on the qualities of such threats and the quality of the situation in which the animal finds itself (e.g. a nest with pups, a territory, a predator etc) an animal may decide to fight, defend, flee or show intermediate or ambivalent behaviour (Baerends, 1973). The three categories mentioned constitute a continuum of agonistic activities with on one pole attack (fight) and on the other pole flight. This ethologically derived scale to distinguish agonistic behaviours has recently been attributed to a new area of behavioural or psychopharmacology, viz. ethopharmacology. This new approach in behavioural pharmacology uses ethological principles to

describe the effects of pharmacological manipulations on animal (an human) behaviour (Miczek, 1987; Olivier **et al.**, 1987a). This branch in particular has been strongly evolved in the study of agonistic behaviour (cf. Miczek, 1983; Miczek **et al.**, 1984; Olivier **et al.**, 1987a).

The present contribution demonstrates the power of an ethological approach to study the behavioural effects of selected serotonergic drugs and benzodiazepines (BDZ) (including agonists, antagonists, inverse agonists)in several aggression paradigms in rats. One animal model for the study of agonistic behaviour, resident-intruder aggression, will serve as prototype to describe ethological methodologies and experimental results of treatment with serotonergic drugs.

Subsequently other animal models, i.e. colony aggression (including hierarchical relations), maternal aggression and hypothalamic aggression will be described and the effects of treatment with benzodiazepines and drugs affecting serotonin (metabolism) will be given. Special emphasis will be focussed on the specificity of behavioural changes and the conditions which determine the eventual drug effects. In the discussion, a summary and hypotheses about the role of 5-HT (receptors), BDZ (receptors) and their possible interaction in agonistic behaviour will be outlined.

Agonistic Behaviour in the rat

Resident-Intruder aggression in rats: behavioural description.

If a male rat is housed (with a female) in a large seminatural environment for some time, this resident will attack strange male intruders (Adams, 1976; Blanchard and Blanchard, 1977; Miczek, 1979; Olivier, 1977). The attacking male performs a complete agonistic repertoire including both appetitive and consummatory behaviour. Aggressive behaviour may consist of searching (patrolling), approach, investigate, threat, fight, chase and dominant posturing. The nature of such interactions between an attacking resident and an

opponent heavily depends amongst others on the quality of the intruder, especially its age (weight) (Flannelly and Flannelly, 1985; Lore and Takahashi, 1984) and hormonal status (Flannelly and Thor, 1978) and the residents aggressive experience (Flannelly **et al.**, 1984; van de Poll **et al.**, 1982). Since these behaviours contain elements of approach and retreat, or attack and flight, it is often referred to as agonistic behaviour (cf. Dixon and Kaesermann, 1987).

It is important to realise that these aggressive acts do not occur randomly. Techniques have been developed to describe the sequences of behaviour and to interpret patterns of agonistic behaviour. In the following paragraphs this methodology and the subsequent results will be shown.

In an extensive ethological study into the hypothalamic brain mechanisms involved in agonistic behaviour of male rats, the basic structure of the behaviour of the residential male against a naive strange intruder during a 10 min-encounter in the territory was determined.

For this purpose 29 behaviour elements were defined to adequately describe the (agonistic) behaviour performed (cf. Olivier, 1977; Olivier **et al.**, 1983). Sequential analyses were made on a total of 210 observations of 10-min encounters. After preparing a transition-matrix of all following and preceding elements, a single-link cluster analysis was applied leading to similarities, which indicate the strength of temporal coupling of elements (for methods see Olivier, 1977; 1981; Olivier **et al.**, 1983).

Figure 1 shows the resulting picture representing the "behavioural struture".

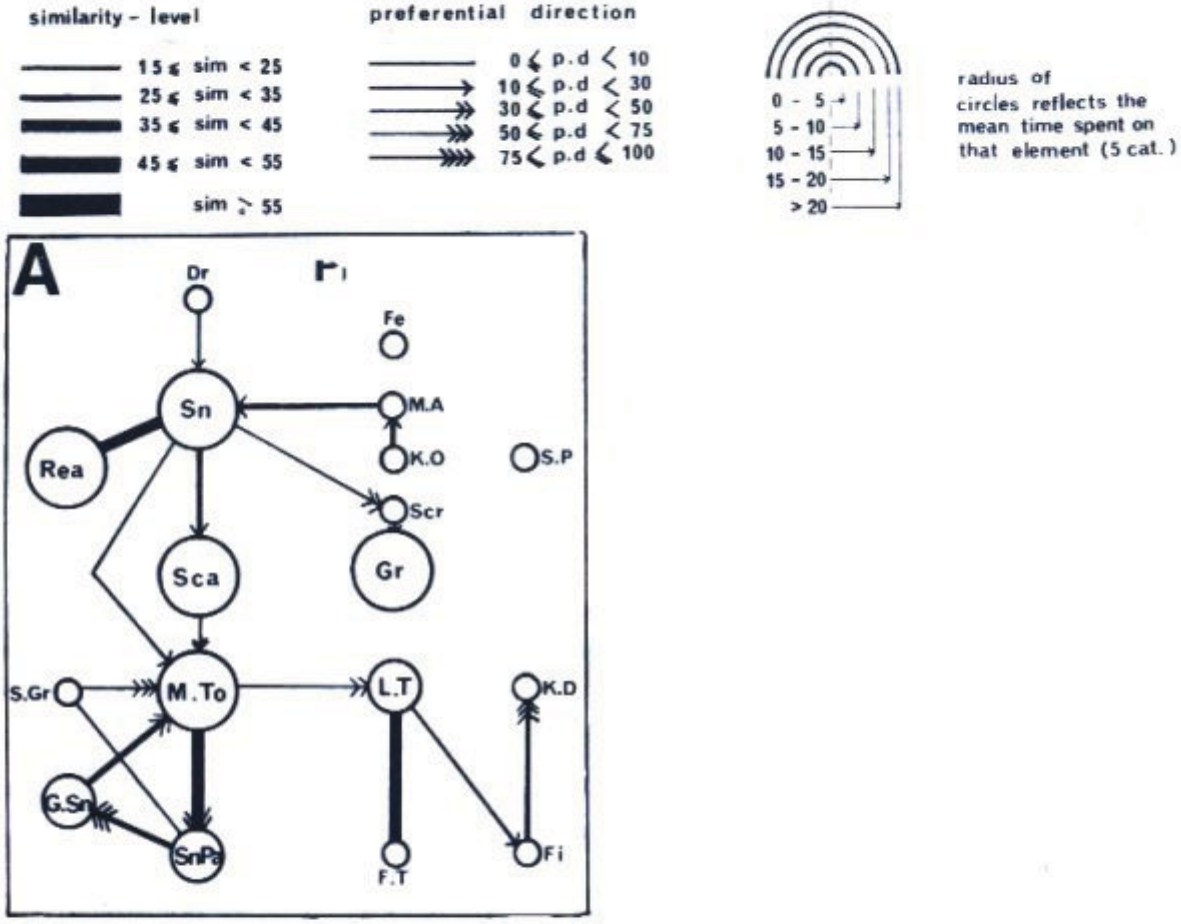

Fig. 1 - Structure of the behaviour (similarities and preferential directions) of untreated male territorial rats when confronted with male opponents. See text for explanations. Sn = sniffing; Dr = drinking; Rea = rearing; Fe = feeding; M.A = moving away; K.O = keeping off; Scr = scratching; Gr = grooming; S.P = submissive posture; Sca = scanning; S.Gr = social grooming; G.Sn = genital sniffing; M.To = moving towards; SnPa = sniffing partner; L.T = lateral threat; F.T = frontal threat; Fi = fighting; K.D = keeping down.

In the figure several behavioural elements are depicted as circles, the larger the circle the more time the animals spent on that particular behaviour. The similarity values represent the transitions (preceding and/or following) between acts; the thicker the line, the higher the similarity. The sequence between elements is represented by the preferential direction, which indicates the principal flow of the behaviour. A high "Preferential Direction" measure (PD) indicates that the behaviour is primarily unidirectional (see legend for further explanation). In this way the behaviour can be presented as a "flowdiagram" of territorial agonistic behaviour in which two main components can be discerned, viz. non-social activities and social activities. The former consist of exploration and body care, the latter of social interest (Introductory Social behaviour: Moving towards, Genital Sniffing, Sniffing of the partner and Social Grooming), Aggressive (Offensive) behaviour (Lateral Threat, Frontal threat (Upright), Fighting and Keeping Down) and Avoidance/ Defensive behaviour (Moving Away, Keeping Off, Submissive posture).

The main streams of behaviour are delineated in such a scheme, showing that behaviour in a resident/intruder situation is not randomized, but follows definite rules. Of course the present scheme only shows the behaviour of the resident animal, which takes initiatives and performs offensive aggression. On the other hand, the intruder tries to evade as much as possible and, if attacked, tries to defend itself optimally. Therefore, agonistic behaviour is **interactive**, in which the behaviour of both partners depends on that of the other. In the resident-intruder (or territorial) situation a clear distinction can be made into an offensive behaviour pattern, displayed by the resident, and a defensive-flight pattern, displayed by the intruder, displayed by the resident, and a defensive-flight pattern, displayed by the intruder. It appears that this distinction within agonistic behaviour (offense/defense-flight) is particularly reflected in certain behavioural elements and

patterns.

Table 1 gives an overview of characteristics typically belonging to one of the subdivisions of agonistic behaviour in the rat.

TABLE 1

	Agonistic behaviour	
Behavioural element	Offense	Defense/Flight
Lateral Threat	+	-
Upright Posture	+	+
On Top	+	-
Jump Attack	+	+(!)
Chase	+	-
Attack	+	-
Approach	+	-
Crouch	-	+
On back	-	+
Evade	-	+
Flight	-	+
Marking	+	-
Ultrasonics (20-30 kHz)	-	+
Piloerection	+	-
Teethchatter	+	-
Primary Bite target	back(+ head)	snout

With the exception of Upright Posture (Offensive or Defensive Upright) and Jump Attack, which may occur both in attacking and defending animals (cornered animal), most elements are largely confined to one of both categories. However, elements shown cannot be taken as isolated units. Piloerection is almost always occurring during most offensive aggressive elements, as is teethchattering. Approach is a very dominant feature of offensive behaviour, which is

strongly characterized by initiative. This also demarcates the difference of Upright Posture in an attacking and defending animal. The former is accompanied by approach, piloerection and initiative, the latter is purely a defending reaction upon the offensive strategy of the attacker. The same holds for jump attack, which is sometimes performed by a cornered animal which has no possibility to escape. In that case, the jump attack is directed towards the snout of the attacker (not necessarily a conspecific), and is accompanied by audible sounds (Blanchard and Blanchard, 1981). A jump attack by an attacking animal may also occur within the context of an offensive strategy, without producing sounds, not directed at the snout but with the intention to expel the intruder from the territory (Blanchard and Blanchard, 1981; Kruk **et al.**, 1984).

In this example, a relative crude behavioural description has been given. Several more refined ethograms, or parts of ethograms have been defined and used, leading to more or less complicated descriptions of agonistic behaviour. In all these studies some interpretation is given to a behaviour. Ethological analysis methods are suitable to unravel the potential significance of an element. Lateral threat or display is an example which serves to underline the problems and possibilities of behavioural analysis.

In the classical works of Grant, Silverman, MackIntosh and Chance (Grant, 1963; Grant and Mackintosh, 1963; Silverman, 1965; Chance, 1968) a rather extensive list of elements occurring in the agonistic repertoire of rats was presented. Lateral threat was used by them in two senses; offensive sideways (aggression) and defensive sideways (flight-submission), indicating that this element can be motivated by at least two sets of opposing causal factors, labelled aggression and flight (Grant, 1963; Silverman, 1965) or offensive and defensive (Lehman and Adams, 1977). On the other hand, several authors consider Lateral threat as a purely offensive element, i.e. motivated only by aggression (offense) (Blanchard and Blanchard, 1977; Miczek and Krsiak, 1979; Adams, 1980; Olivier, 1981).

In an attempt to unravel the possible ambivalent nature of lateral threat (or lateral display), Van der Poel **et al.**(1984) studied the occurrence of fights between a territorially housed male rat and a naive male intruder using slow-motion video analysis. Based on the timing of lateral display, the nature of the display, the accompanying behaviour and the sequential association with other behaviour patterns, these authors conclude that two opposing tendencies are present in the lateral display. One tendency to approach the intruder (motivated by aggression) and a tendency to evade the intruder (motivated by defence/flight). The simultaneous presence of two opposing but mutually excluding tendencies leads to a behavioural conflict as expressed in the orientation of the display, circling around the intruder, intention movements and signs of strong autonomic arousal (piloerection and teethchattering).

These examples of motivational backgrounds of Lateral Display or Threat indicates the difficulty of studying agonistic behaviour in the context of ethopharmacology. At the same time it indicates the importance of detailed observation and recording of the ongoing behaviour, if possible with video-recording, because this gives the possibilities to re-score the behaviour or even to re-define behavioural elements if necessary. Fig. 2 shows our experimental set-up to record ongoing behaviour, and illustrates the recording of behaviour and sounds (both audible and ultrasonic).

Ultrasonics and the behaviour are mixed directly on the video-tape and are also made audible by lowering the frequency by a factor 10. This enables us to record in detail which animal produces sounds and during which behaviour.

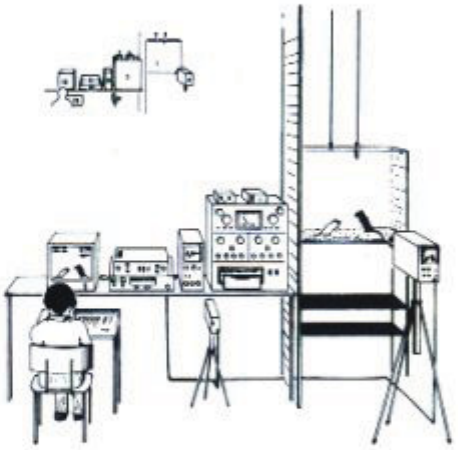

Fig. 2 - Schematic picture of the experimental set-up used to record animal agonistic behaviour in our laboratory. 1 = cage; 2 = ultrasonic microphone; 3 = audio microphone; 4 = infrared camera; 5, 6, 7 = ultrasonic-sonic conversion and measurement; 8 and 9 = oscilloscope with ultrasonic signal mixed on the video via camera; 10 and 11 = video recorder; 12 and 13 = monitor and computer terminal.

Drug studies in resident-intruder aggression

The resident-intruder paradigm has been introduced in pharmacology only quite recently (Miczek, 1979; Olivier, 1981; Olivier **et al.**, 1984a), but has firmly established its role in the pharmacological research of aggressive behaviours (cf. Miczek, 1987; Olivier **et al.** 1987b).

The paradigm is very sensitive for anti-aggressive activities of psychoactive drugs and clearly indicates specificity of such activities (Olivier **et al.** 1984a,b; Olivier **et al.** 1986; 1987b), thus showing whether sedation, psychostimulation, muscle relaxation or sensoric-motoric disturbances interfere with the behavioural performance. Also the paradigm is sensitive to pro-aggressive actions of drugs, as e.g. occurring after low dosages of benzodiazepines (Mos **et al.** 1987a; Mos and Olivier, 1987; Mos and Olivier, 1988).

In this resident-intruder paradigm the serotonergic compounds TFMPP (a mixed 5-HT 1B,1C-agonist), fluprazine (a weak 5-HT 1-agonist), eltoprazine (a mixed 5-HT 1A/1B agonist), fluvoxamine, a specific 5-HT uptake blocker, buspirone, a 5-HT 1A agonist and the benzodiazepine agonist oxazepam were tested. Although in the previous paragraphs the depth of analysis dominated, this is not always an easy representation for the uninitiated. To illustrate the overall effects of drugs on agonistic behaviour, we have therefore summarized the several behavioural elements in behavioural categories.

Fig. 3 shows the effects of fluprazine on 7 broad categories of agonistic behaviour, viz, inactivity, exploration, body-care, sexual behaviour, social interaction (ISB), aggression and avoidance. Under vehicle conditions the piecharts in the right part of fig. 3 show the percentage of time spent on these different categories. Aggression amounts to approx. 9%, but exploration (29%) and social interest (26%) together constitute the bulk of time spent by the resident during the agonistic interaction. Fluprazine (an early serenic - Olivier **et al.**, 1984a,b; Bradford **et al.**, 1984; Van der Poel **et al.**, 1982) dose-dependently reduced aggression, till 20% of the vehicle-level (at 20 mg/kg p.o.) Concomitantly, social interest (ISB) was only reduced to approx. 80%, which was significantly different from control. The effects on aggression and ISB are further illustrated in fig. 4, showing the effects of fluprazine on the individual elements. Fluprazine (DU 27716) affect all aggression elements in a dose-dependent way, whereas some elements of Social Interest are not (e.g. Sniffing at partner or nosing) or just slightly decreased.

TFMPP (meta-trifluoromethylphenylpiperazine), a 5-HT1 agonist with high affinity for 5-HT1B and 5-HT1C-receptors (Olivier **et al.**, 1988) also reduced aggression in a dose-dependent way (fig. 5), again without affecting social interest. Exploration was increased, body care and avoidance unaffected, whereas inactivity showed some enhancement

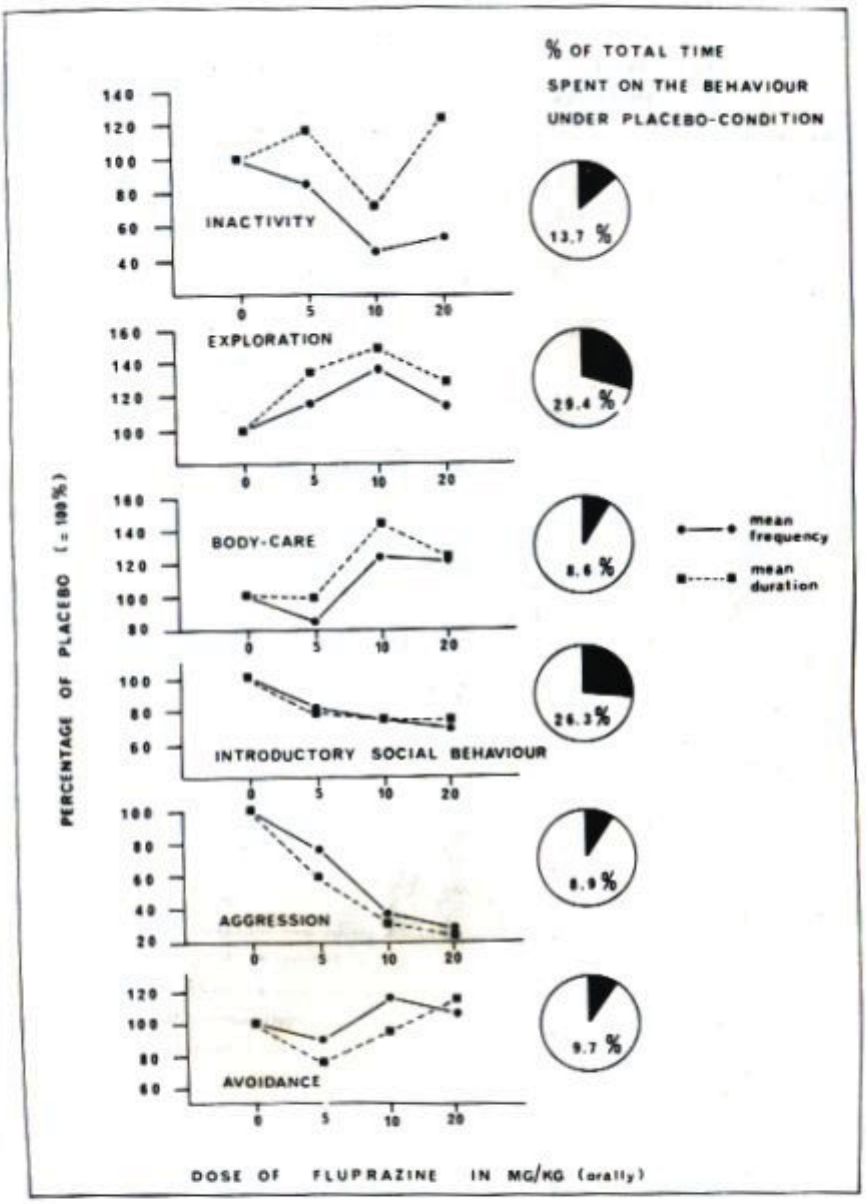

Fig. 3 - Effects of fluprazine hydrochloride (mg/kg, p.o.) on seven behavioural categories in the resident-intruder paradigm.

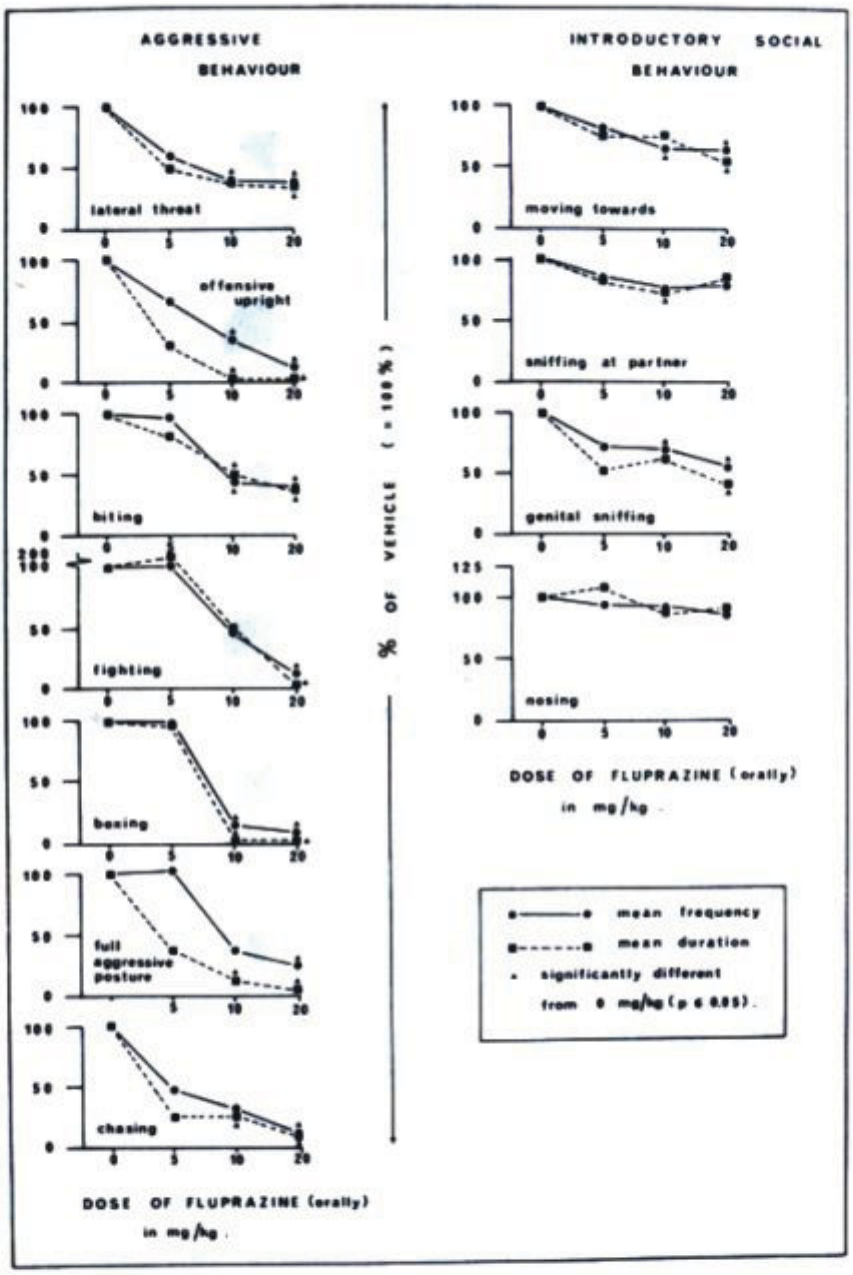

Fig. 4 - Effects of fluprazine hydrochloride (mg/kg, p.o.) on 7 aggressive behaviour elements and four social interest (ISB) elements in the resident-intruder paradigm.

(particularly in time).

Such a pattern was also observed after eltoprazine (DU 28853), a serenic drug with a mixed 5-HT1A/1B/1C agonistic character (Olivier **et al.**, 1989) (Fig. 5).

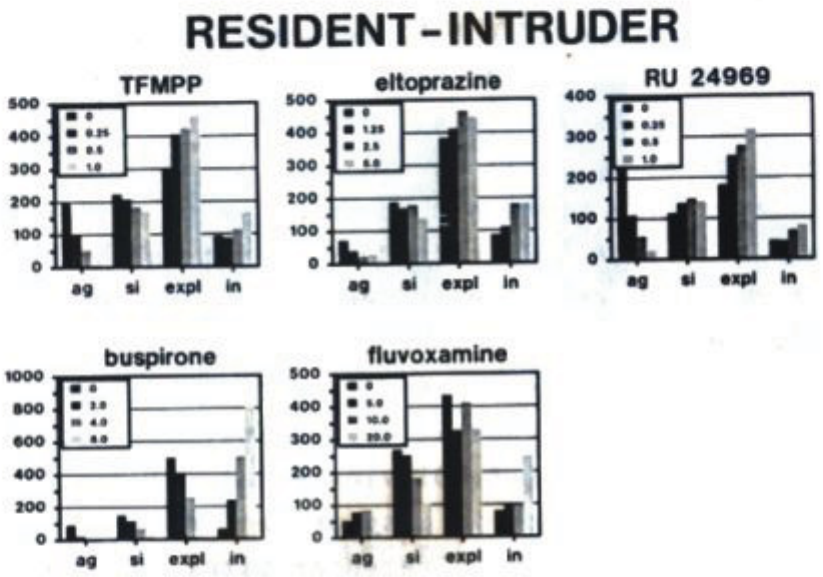

Fig. 5 - Effects of meta-trifluoromethylphenyl piperazine (TFMPP) (mg/kg, i.p.), eltoprazine hydrochloride (DU 28853) (mg/kg) p.o.), RU 24969 (5-methoxy-3-(1,2,3,6-tetrahydro-4-pyrimidyl) indole succinate) (mg/kg, i.p.), buspirone (mg/kg, i.p.) and fluvoxamine (mg/kg, i.p.) on four behavioural categories in the resident-intruder paradigm. Ag = aggression, si = social interest, expl = exploration, in = inactivity.

RU 24969 (5-methoxy-3-(1,2,3,6-tetrahydro-4-pyrimidyl) indole succinate), a potent 5-HT1 agonist (mixed 5-HT1A/1B) also strongly reduced aggression concomitant with increases in social interest, exploration, avoidance and inactivity (fig. 5). Self care was unaffected. RU 24969 has a strong "stimulatory" effect in this aggression model which is

especially marked in exploration (more than 100% increase at 1 mg/kg compared to vehicle).

Buspirone, a 5-HT1A agonist with considerable dopaminergic properties (Olivier **et al.**, 1984a; 1989) had strong anti-aggressive effects but this was clearly associated with heavy sedation, as indicated by concomitant decreases in social interest, exploration, avoidance and a strong increase in inactivity (fig. 5).

Fluvoxamine, a specific 5-HT reuptake blocker (Claassen **et al.**, 1977) reduced aggression but not in a very specific way as indicated by decreases in social interest and increases in inactivity (fig. 5).

These data on serotonergic compounds with differential effects on subsites of 5-HT receptors, point to the specific involvement of 5-HT1 receptors in resident-intruder aggression. More notably, especially the 5-HT1B subtype seems to be involved in the modulation of this kind of behaviour without interference with social capacities, sensory/motoric disturbances or sedation (cf. Olivier et al., 1987b; 1988). In these papers cited evidence is also presented which shows that antagonists of 5-HT2 and 5-HT3 receptor sites are unlikely to be involved in the modulation of aggressive behaviour.

Besides serotonergic involvement in the modulation of aggression there is also abundant evidence that benzodiazepines may influence agonistic behaviour (cf. Mos and Olivier 1987; Mos **et al.**, 1987), but in contrast to serotonergic compounds there is not only a reduction of aggression.

As an example, the effects of oxazepam, a benzodiazepine agonist are shown in the resident-intruder paradigm (fig. 6).

Oxazepam enchanced aggressive behaviour. This pattern is also observed after other benzodiazepines, like chlordiazepoxide, alprazolam and diazepam (Mos and Olivier, 1989). In general, biphasic dose-response curves are observed. At lower doses aggression is enchanced and at higher doses aggression is returned to baseline or even below that. Although the latter is not observed here for oxazepam, it

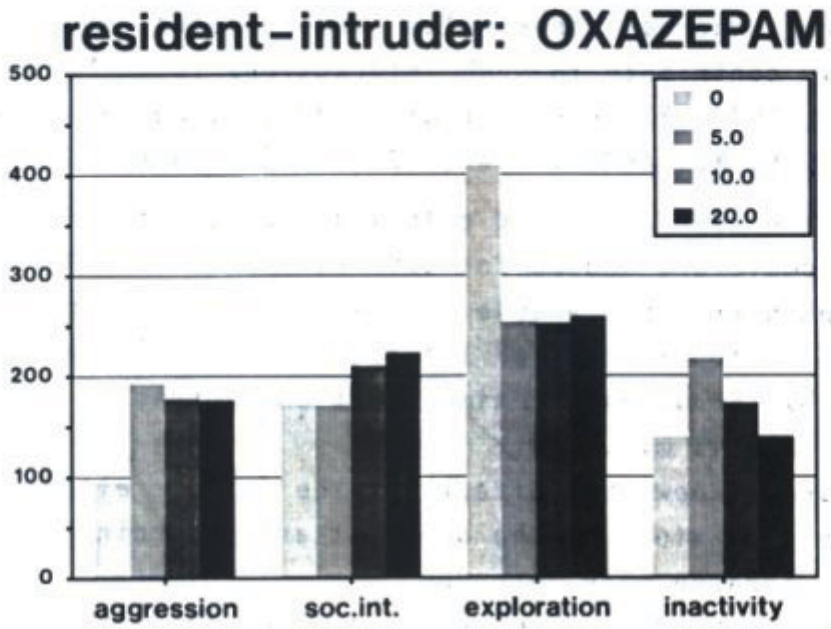

Fig. 6 - Effects of oxazepam (mg/kg, i.p.) on four behavioural categories in the resident-intruder paradigm.

probably would occur at higher doses. These reductions are probably caused by other effects induced by benzodiazepines like muscle relaxation and sedation which parallel or cause the decrease in aggression.

Colony aggression in male rats

When a group of rats, males and females, is housed in large environments, a colony emerges in which animals with differential roles develop (Blanchard and Blanchard, 1977, 1981; Blanchard **et al.**, 1977).

A typical dominant or α-male exists which can be distinguished as such by different measures, one of these being

the predominant attack against a strange male introducted into the colony (Mos **et al.**, 1987a; Mos and Olivier, 1988). The α-male performs the bulk of aggressive behaviour towards such an intruder, whereas other males (subordinates) only marginally contribute to such interactions (Blanchard and Blanchard, 1977; Blanchard **et al.**, 1977; Timmermans 1978; Mos **et al.**, 1985; Mos and Olivier, 1988; Dijkstra **et al.**, 1984).

We have used a limited colony-situation in which two males and one female are housed for several months in a large cage. Weekly intruder tests enable to follow the development over time (one to two months) of the emergence of a clear α-male, measured by the amount of time spent on aggression towards an intruder in a 15 min. test.

Figure 7 shows the effects of the serenic eltoprazine on aggressive behaviour of the dominant and subordinate rat against a strange male intruder.

Eltoprazine dose-dependently reduced the aggressive behaviour of the dominant and the subordinate towards the intruder, although the dominant male seems to be more heavily affected. The joint aggressive behaviour (D + S) against the intruder is also decreased as is the (normally already very low) aggressive interaction between the two colony members (D vs S).

In contrast, chlordiazepoxide (CDP), a benzodiazepine agonist, has, at least at doses of 5 and 10 mg/kg p.o., aggression enhancing effects (fig. 8A).

This effect is evident for the dominant male, but even more pronounced in the subordinate male and in the joint aggression of the dominant and the subordinate against the intruder. In the latter case, even at 20 mg/kg, a dose which clearly reveals the muscle-relaxing properties of CDP, a strong enhancement of aggression is still noted (see Fig. 8B for % changes).

Chlordiazepoxide especially affects the subordinate's behaviour, probably by reducing the normal inhibitory of the

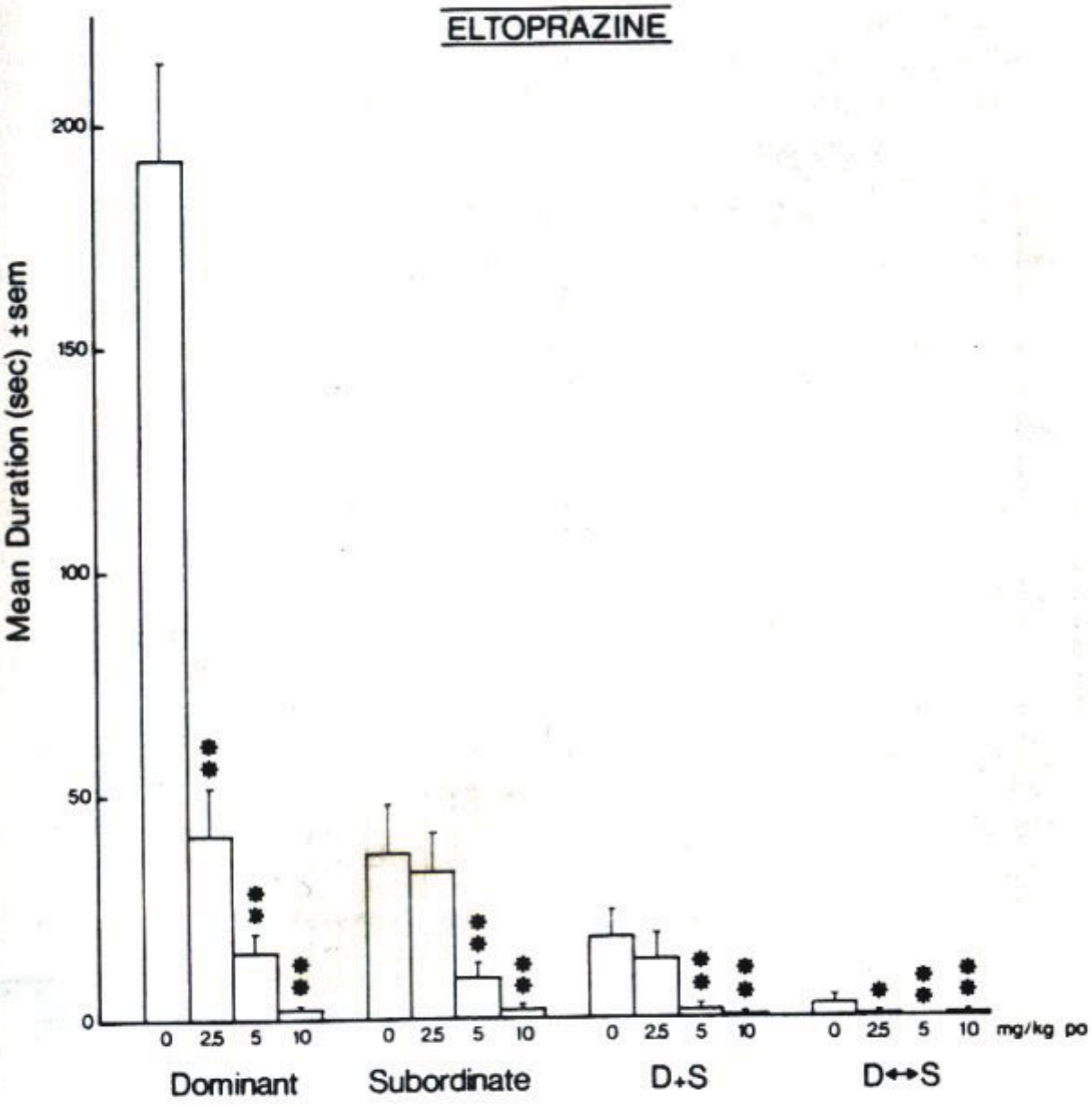

Fig. 7 - Effects of eltoprazine (DU 28853) (mg/kg, p.o.)on the aggressive behaviour of the dominant (D), the subordinate (S) and the dominant + subordinate together (D + S) versus a male intruder in a mini-colony situation. DvsS represents the aggression between the two residents. *: $p < 0,05$; ** $p < 0,01$ significantly different from vehicle.

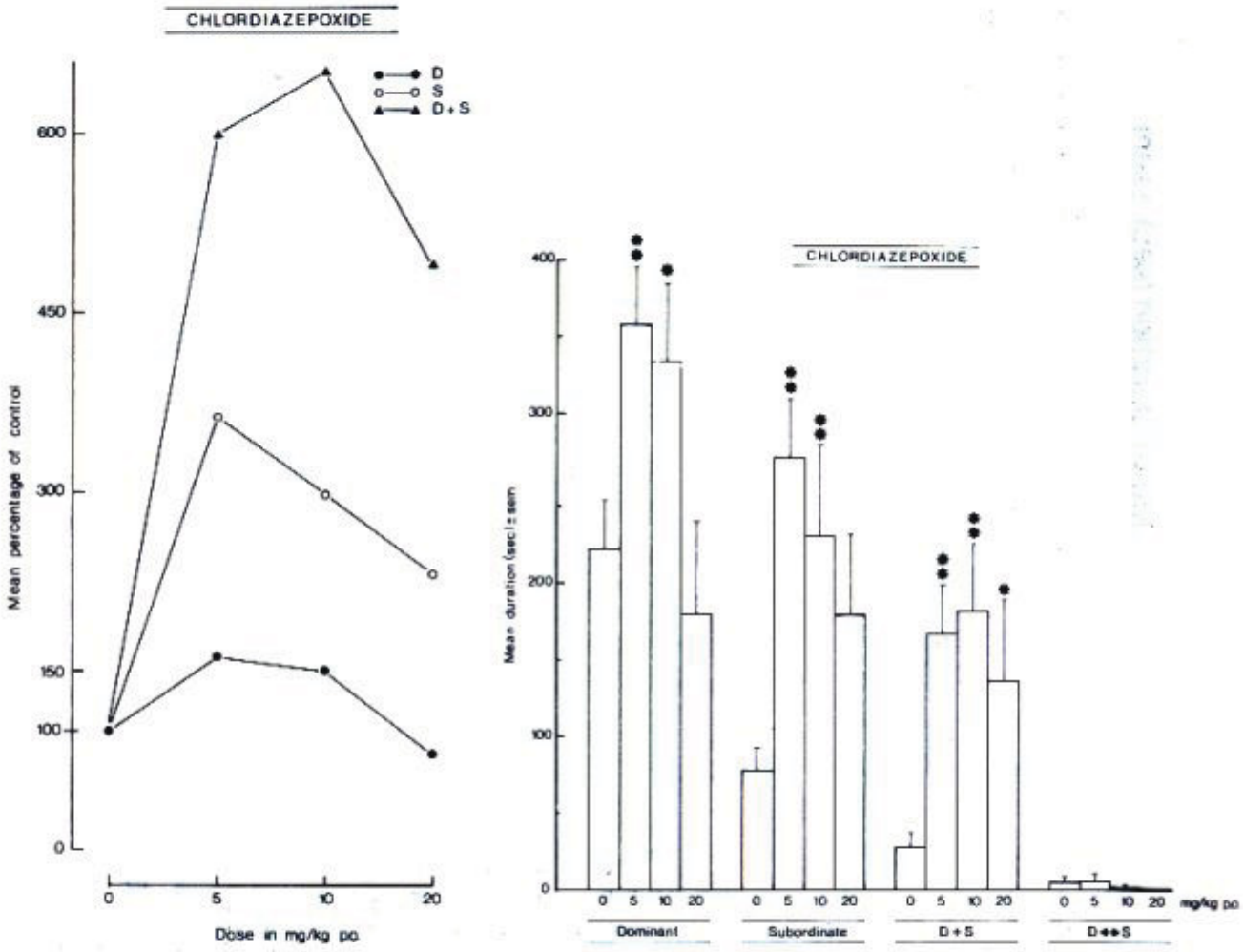

Fig. 8 - (A) Effects of chlordiazepoxide (mg/kg, p.o.) on the aggressive behaviour of the dominant (D), the subordinate (S), the dominant and the subordinate together (D + S) versus a male intrude in a mini-colony situation. DvsS represents the aggression between the two residents. *:P < 0,05; **: p < 0,01; significantly different from vehicle.
(B) Data are now expressed as percentage of vehicle (0 mg/kg).

α-male on the subordinate. This indicates that a subordinate's level of aggression is state-dependent; removal of the α-male or treatment with benzodiazepines (anxiolytics) may reinstall the original level of aggression.

That the α-male is also susceptible for the aggression-enhancing effects of BDZs is remarkable in the sense that apparently these animals are also somewhat inhibited in their aggressive behaviour. This may be due to the presence of a subordinate rival or to other inhibitory processes of which we are not aware.

Treatment of colony-members with alcohol (0,5, 1 and 2 mg/kg po) has no effects on aggressive behaviour of either member of the colony (Mos and Olivier, 1988). These data illustrate the attractiveness of the colony situation for the study of drug effects on complex and hierarchical behavioural structures. Both simultaneous decreases in aggression in both members as well as role-dependent increases in aggression can be observed. Therefore, this aggression paradigm needs far more investigations using the maximal possibilities of such a test model, viz. combined or separate treatment of dominant and subordinate males.

Maternal Aggression in lactating rats

Lactating females defend their pups and nest area against threatening objects, e.g. strange conspecifics. Lactating female rats of different strains attack male conspecific instruders with short latencies using a high intensity form of attack, primarily targeted at the head and upper back (Olivier and Mos, 1986a,b). Figure 9 shows some parameters of the aggression and other behaviours performed by a lactating female rat against a male conspecific during a 5 min. test.

For testing of experimental drugs, the lactation period between 3-12 days after birth was used as this appeared a relatively stable period to perform aggression tests using each female as its own control (Olivier **et al.**, 1985, 1986; Olivier

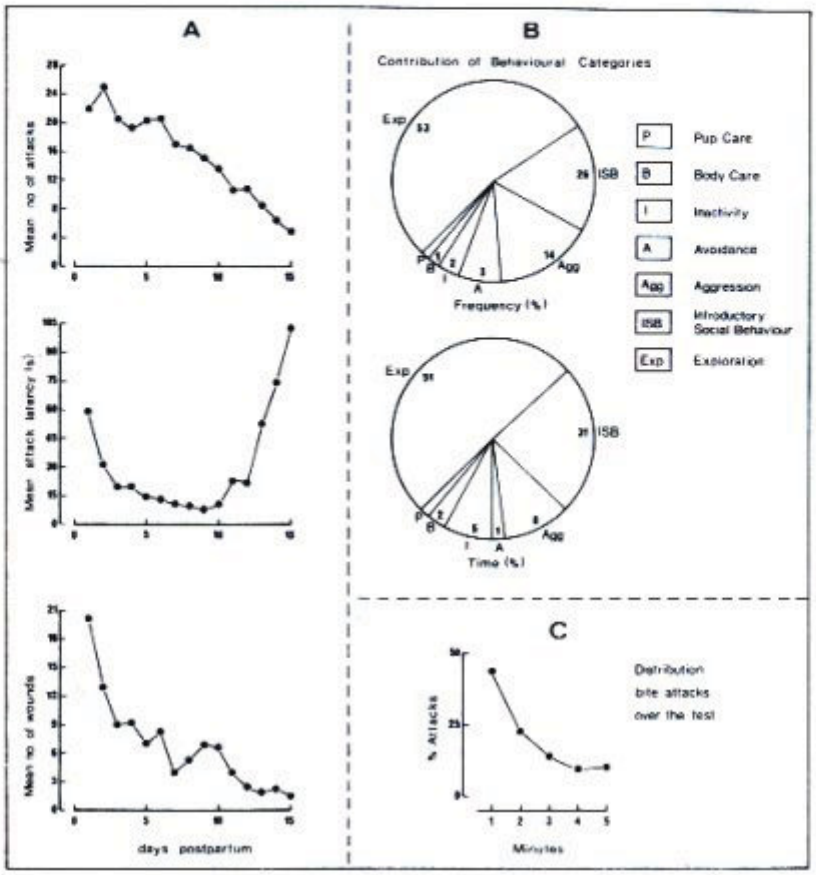

Fig. 9 - Maternal aggression of lactating rats over the first 15 potpartum days. The left panel (A) shows the mean number of attacks (top), the mean attack latency (middle) and the mean number of wounds (bottom) inflicted upon the intruders over the first 15 postpartum days in 5 min aggression tests. The right panel (B) shows the distribution of the different behavioural categories over the total observation period. Both the frequency (top) and the duration (bottom) distribution are shown. The distribution (in %) of the bite attacks within one test period of 5 minutes is shown in C (right bottom).

and Mos, 1986a, b). Detailed studies into the behavioural structure of this maternal aggression revealed a period of fairly stable aggression levels during day 3-12 of the post-partum period (Olivier and Mos, 1986a).

Moreover, these studies showed the offensive motivation of the female. She directs much of her behaviour towards the

intruder, takes the initiative to attack while her behaviour is relatively independent of the qualities (male, female or castrated) of the intruder and its behaviour (Mos **et al.**,1987b; Mos and Olivier, 1987; Mos **et al.**, 1989).

Several drugs were tested in this maternal aggression paradigm, using 5 min test periods. Each female was repeatedly tested on alternate days between days 3 to 12 postpartum. Only the mean number of bite attacks/minute is given, a measure corrected for the latency to attack for the first time. Fig.10

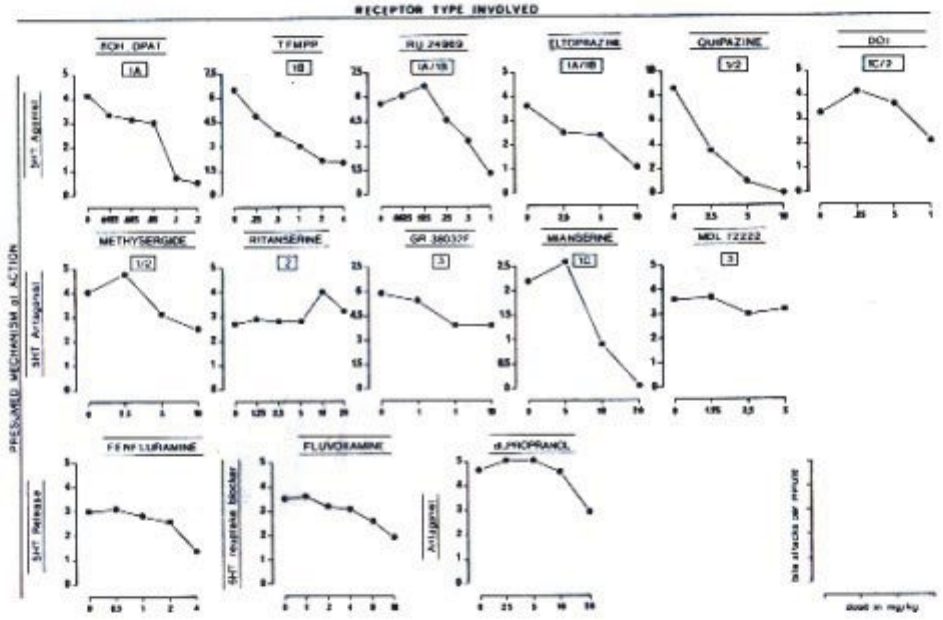

Fig. 10 - Maternal aggression in rats. The mean number of bite attacks/minute (± SEM) is shown for several serotonergic drugs.

shows the effects of several serotonergic drugs, showing that a considerable number of such drugs inhibits aggression, although there are vast differences between drugs (cf. Olivier **et al.**, 1987b). In general 5-HT agonists (1A, 1B, 1C) reduce aggression, whereas 5-HT antagonists (5-HT1, 5-HT2 or 5-HT3) either have no influence or reduce it in a nonspecific way (mianserine).

Detailed ethological studies, involving the complete behavioural repertoire, showed that most drugs reducing aggression did so in a behaviourally nonspecific way, e.g. by sedation (cf. Olivier **et al.**, 1987b, 1989). These detailed analyses strongly suggest that the 5-HT1B receptor might be involved in a specific modulation of aggressive (offensive) behaviour (cf. Olivier **et al.**, 1987b, 1989).

Studies on benzodiazepine agonists, antagonists and inverse agonists in maternal aggression indicated that BDZ-agonists have pro-aggressive effects in this model. Chlordiazepoxide, diazepam, oxazepam and alprazolam exerted biphasic effects on aggression (fig. 11), enhancing it at lower

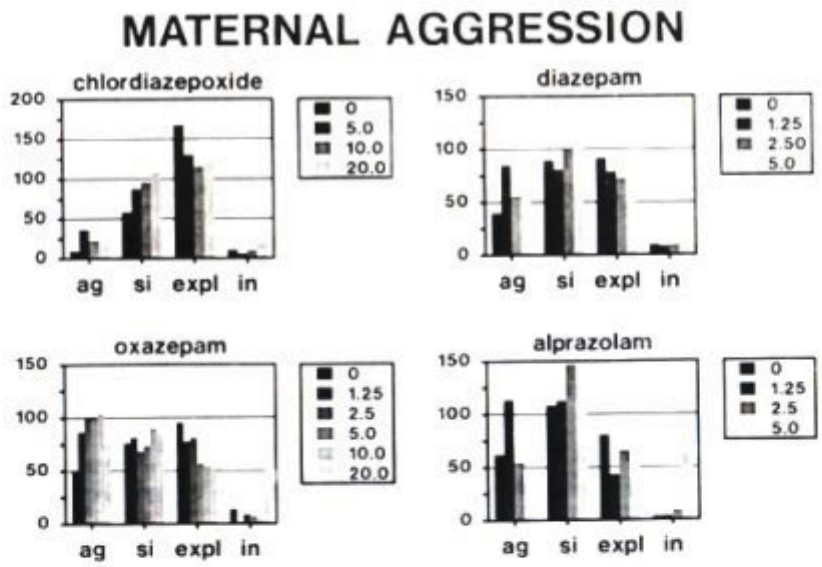

Fig. 11 - Effects of chlordiazepoxide (mg/kg, p.o.), diazepam (mg/kg, i.p.), oxazepam (mg/kg, p.o.), and alprazolam (mg/kg, p.o.) on four behavioural categories in maternal aggression in lactating female rats. Ag = aggression, si = social interest, expl = exploration, in = inactivity.

doses and reducing it at higher doses, especially alprazolam which completely reduced aggression at the highest dose used, and replaced it by inactivity (sedation) (cf. Olivier **et al.**, 1985; Mos **et al.**, 1987a; Mos and Olivier, 1987).

The BDZ-antagonist Ro15-1788 (up to 20 mg/kg ip) had no effects on aggressive behaviour (Mos and Olivier, 1986), whereas the inverse agonist β-CCE (fig. 12) decreased aggression at the rather high dose of 40 mg/kg, but this coincided with nonspecific effects (inactivity enhanced; see Mos **et al.**, 1987a). Although this anti-aggressive activity is not specific, the data obtained with BDZ-ligands indicate that the BDZ-receptor may play a bidirectional role in aggressive behaviour of lactating females.

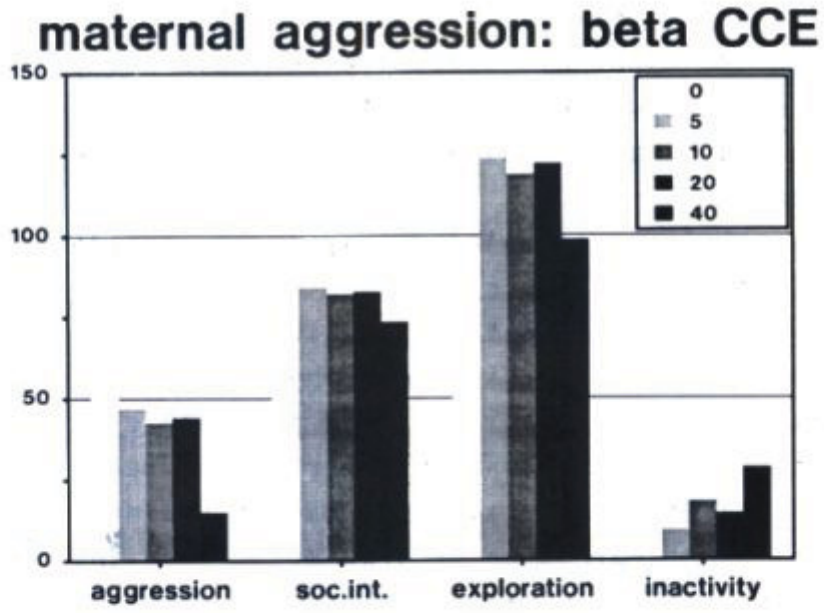

Fig. 12 - Effects of beta CCE (mg/kg, i.p.) on four behavioural categories in maternal aggression in lactating female rats.

Hypothalamic Aggression

Electrical brain stimulation-induced attack (EBS) can be evoked in male and female rats by electrical stimulation of circumscript localisations in the hypothalamus (Kruk **et al.**, 1979; 1983; 1987).

This socalled hypothalamic aggression resembles many features also occurring in territorial, maternal, offensive and defensive behaviour (Kruk **et al.**, 1979; Kruk and Van der Poel, 1980). Electrical stimulation of the neural substrates in the hypothalamus evokes several forms of attack, both with low and high intensity components (Kruk **et al.**, 1987), largely depending on stimulating with different current intensity. On the other hand, hypothalamic aggression is quite different fromother aggression types, e.g. in the factors controlling fighting (gender and qualities of the opponent, occurrence in a strange environment, purely stimulation-bound). Kruk **et al.** (1987) suggest that activation of the socalled "aggressive area" in the hypothalamus activates brain mechanism necessary to perform behaviour adequate for attack. Such a mechanism may be involved in all kinds of agonistic behaviour and in predation.

Hypothalamic stimulation may evoke, besides aggression, several other behaviours like teeth chatter, locomotion, switch-on (self-stimulation) and swith-off behaviours (Kruk **et al.**, 1983, 1984). Detailed mapping of the respective neural substrates involved has indicated that one electrode tip may activate many independent but overlapping neural systems, each system with its own behavioural output (Lammers **et al.**, 1987, 1988a,b). Determination of drug effects on these independent systems may give indications about the specificity of a drug's action (cf. Olivier **et al.**, 1986; Van der Poel **et al.**, 1982).

The effects of four serotonergic drugs and one benzodiazepine agonist have been studied on this form of aggressive behaviour in male rats using the threshold method described in Kruk **et al.** (1979). Table 2 shows the effects of eltoprazine, fluprazine, fluvoxamine, 8-OH-DPAT and

Table II: Effects of five drugs on thresholds for aggression and locomotion in the EBS–paradigm. Data are expressed as percentage of vehicle (=100%); n.t.= not tested

8–OH–DPAT	0	0.25	0.5 mg/kg	
aggression	100	115	167	
locomotion	100	98	92	
eltoprazine	0	0.25	0.5	1.0 mg/kg
aggression	100	125	147	208
locomotion	100	101	97	95
fluvoxamine	0	10	20	40 mg/kg
aggression	100	145	138	152
locomotion	100	105	117	162
chlordiazepoxide	0	5	10	20 mg/kg
aggression	100	97	86	157
locomotion	100	138	149	166
fluprazine	0	5	10	20 mg/kg
aggression	100	160	148	192
locomotion	n.t.	n.t.	n.t.	n.t.

chlordiazepoxide on current thresholds for aggression and locomotory behaviour. The latter can be measured using stimulation of the same electrode in a solitary environment measuring the locomotory behaviour (cf. Van der Poel **et al.**, 1982).

Eltoprazine and fluprazine reduce aggression (measured by enhanced current thresholds) without concomitant inhibitory effects on locomotion; eltoprazine even reduces locomotion somewhat.

Quipazine nonspecifically reduced aggression, as locomotion is concomitantly reduced. The same, although to a less degree, holds for fluvoxamine. 8-OH-DPAT has no effect on aggression although locomotion is even decreased. Chlordiazepoxide has no influence on aggression, although at the highest dose, due to muscle relaxation, locomotion is even decreased (enhanced threshold).

The results in this aggression paradigm again strongly suggest that specific reduction of aggressive behaviour is modulated by 5-HT1B receptors, because a specific 5-HT1A (8-OH-DPAT) agonist has no influence on aggression while other, less specific serotonergic compounds have no specific influence. The important role for 5-HT1B is further supported by data on TFMPP, one of the most specific 5-HT1B agonist presently available (Kruk **et al.**, 1987; Olivier and Mos, 1988a).

Discussion

Serotonin

The experiments have demonstrated that serotonergic drugs, if anything, reduce aggression, whereas benzodiazepine agonists enhance, at least at low dosages, aggression. Till now, we have never observed an increase in aggression after treatment with serotonergic compounds. Whether this is due to the absence of specific serotonergic antagonists (e.g. for 5-HT1B and 5-HT1A) or agonists (5-HT2, 5-HT3) is unclear. Moreover it

is difficult to predict whether the serotonergic system, or subdivisions of it, has a certain "tone", which upon blocking might result in pro-aggressive effects.

The general notion that 5-HT inhibits aggression comes from indirect studies, using rather crude manipulations to change 5-HT activity, e.g. depletion by pCPA, neurotoxin lesions by 5,7-DHT or adding precursors of 5-HT (cf. Miczek and Donat, 1989). Also, studies correlating 5-HT turnover with aggression have been used to establish a role for 5-HT in aggressive behaviour (cf. Olivier **et al.**, 1987b). All these measures did not reveal an univocal picture of the relationship between 5-HT and aggression. The recent differentiation in 5-HT receptor (sub)sites and their anatomical distribution has further complicated the picture.

The use of agonists (partial) or antagonists to unravel the function of a certain neurotransmitter system is tricky. Especially when a drug exerts mixed agonist/antagonist (or partial agonist) activity, with a considerable variation in intrinsic activity, the effects of such a drug may vary depending on the location where it acts and the experimental situation. A further complication is that most drugs are not "selective", in the sense that, apart from other neurotransmitters, they influence different 5-HT receptors. Moreover, species differences occur, even between related species, such as the rat and the mouse. An example is 8-OH-DPAT, a very specific 5-HT1A agonist, in sexual behaviour. The confusing picture arises of a stimulatory effect on sexual behaviour in male rats (e.g. Ahlenius and Larsson, 1987) and an inhibiting effect in male mice (Svensson **et al.**, 1987). In another drug, RU24969, a mixed 5-HT1A,B and weak 5-HT1C-agonist, an inhibitory influence on sexual behaviour in male rats was found (Oliver and Mos, 1988b). Apparently, in this case an inhibitory 5-HT1B site dominates over the stimulatory 5-HT1A site. Thus caution is needed in the interpretation of receptor subtype mediated behavioural effects.

Such a species discrepancy between mice and rats in male sexual behaviour so far does not occur in aggressive behaviour, neither in male nor in female agression (Flannelly **et al.**, 1985; Olivier **et al.**, 1986; Racine and Flannelly, 1986).

The available evidence we have gathered up to now on the effects of serotonergic compounds has been summarized in tables III and IV.

In these tables the effects of several serotonergic drugs in 7 aggression paradigms have been given, 5-HT1A agonists like 8-OH-DPAT, buspirone, ipsapirone and flesinoxan (Hartog and Wouters, 1988) either have no, or nonspecific decreasing effects on aggression, suggesting that the 5-HT1A receptor is not (specifically) involved in the modulation of aggressive behaviour. Drugs with strong agonistic effects on the 5-HT1B receptor site seem to exert specific anti-aggressive effects in all aggression paradigms studied. Fluprazine, an early serenic and a weak and nonspecific 5-HT1,2 agonist, probably gives rise to the metabolite TFMPP, a potent and specific anti-aggressive drug. Eltoprazine, a new serenic drug (Hartog and Olivier,1988) is also a potent agonist (partial) at the 5-HT1B site, apart from a putative agonistic action on the 5-HT1A site and a weak antagonistic action on the 5-HT1C site (cf. Olivier **et al.**, 1989). Apparently, the effects on the 5-HT1A or 5-HT1C-receptors do not interfere with the specific modulation of aggressive behaviour. RU24969, also a mixed 5-HT1A,B agonist, but more potent than eltoprazine, is less specific than eltoprazine in suppressing aggressive behaviour, primarily due to a strong stimulatory action present in its behavioural spectrum.

The involvement of 5-HT1C, 5-HT2 and 5-HT3 receptors in the modulation of (offensive) aggression is not very likely in view of the effects of DOI (5-HT1C and 2 agonist), quipazine (a potent 5-HT3 antagonist but at the dose used probably a nonspecific ligand), ritanserine (a 5-HT1C and 2-antagonist), mianserine (a 5-HT1C,2 antagonist) and the specific 5-HT3 antagonists (MDL72222 and GR38032F). It is also clear from

Table III Summary of the effects of serotonergic drugs on several aggression paradigms in mice (m) and rats (r).

DRUG	isolation induced aggression (m)	intermale aggression (m)	footshock induced defence (m)	resident-intruder aggression (r)	maternal aggression (r)	EBS (r)	muricide (r)	PUTATIVE 5-HT MECHANISM OF ACTION*
8-OH-DPAT	↓	↓	–	↓	↓	o	o	1A-agonist
Buspirone	o	o	–	↓	↓	–	↓	1A-agonist
Ipsapirone	o	o	–	–	↓	–	o	1A-agonist
Flesinoxan	↓	↓	–	–	↓	–	↓	1A-agonist
TFMPP	↓	(↓)	–	(↓)	(↓)	(↓)	(↓)	1C,1B-agonist, weak 1A-agonist
Eltoprazine	↓	(↓)	o	(↓)	(↓)	(↓)	(↓)	1A,1B-agonist, weak 1C-antagonist
RU24969	↓	↓	–	(↓)	(↓)	–	↓	1A,1B-agonist, weak 1C-agonist
5-Me-O-DMT	↓	–	–	↓	–	–	↓	1A,1C,1B-agonist
Fluprazine	↓	(↓)	(↓)	(↓)	(↓)	(↓)	(↓)	weak 1A,2,1C,1B agonist
DOI	o	–	–	↓	↓	–	o	1C,2-agonist
Befiperide	↓	↓	–	↓	–	↓	↓	1A,2-agonist

(↓): specific behavioural decrease; ↓:nonspecific behavioural decrease; o: no effect; –: not tested. EBS=Electrical brain stimulation-induced aggression.

* : the highest affinity for any of the subtype of 5-HT receptors is indicated first, followed by progressive decreasing affinity for the other subtypes.

Table IV Summary of the effects of serotonergic drugs on several aggression paradigms in mice (m and rats (r).

DRUG	isolation induced aggression (m)	intermale aggression (m)	footshock induced defence (m)	resident-intruder aggression (r)	maternal aggression (r)	EBS (r)	muricide (r)	PUTATIVE 5-HT MECHANISM OF ACTION*
MDL 72222	o	-	-	-	o	-	-	3-antagonist
GR 38032F	o	o	-	-	o	-	o	3-antagonist
Quipazine	o	-	-	↓	↓	-	↓	3-antagonist, weak 1C,2-agonist
Methysergide	o	-	-	-	o	o	o	1,2-antagonist
Ritanserine	o	-	-	-	o	-	o	1C,2-antagonist, weak 2-agonist
Mianserine	↓	-	-	-	↓	-	-	1C,2-antagonist
dl-Propranolol	↓	-	-	(↓)	o	(↓)	↓	weak 1-antagonist
Fluvoxamine	↓	(↓)	-	↓	↓	↓	↓	reuptake blocker
Fenfluramine	↓	-	-	-	↓	-	↓	release

(↓):specific behavioural decrease; ↓:nonspecific behavioural decrease; o no effect; -: not tested. EBS=Electrical brain stimulation-induced aggression.

* : the highest affinity for any of the subtype of 5-HT receptors is indicated first, followed by progressive decreasing affinity for the other subtypes.

these thables that 5-HT antagonists (whether 5-HT1,2 or 3) ever enhance aggression. To further unravel the role of 5-HT in aggression and more specifically those of the different receptortypes, studies with specific agonistst and antagonists are badly needed. Antagonists for 5-HT1A and B are largely lacking, although recent evidence indicates that propranolol and pindolol may act as 5-HT1 antagonists.

These compounds have been shown in particular to behave as 5-HT1A-antagonists as evidenced by antagonizing the serotonin syndrome (Tricklebank, 1985), the hypothermic effects (Goodwin **et al.**, 1985) and the discriminative stimulus (Tricklebank **et al.**, 1987) induced by 8-OH-DPAT. Drug-discrimination studies using TFMPP as discriminative stimulus in rats (Glennon **et al.**, 1984) suggest that the stimulus cues of TFMPP are 5-HT1B-modulated (Cunningham and Appel, 1986; McKenney and Glennon, 1986). Glennon **et al.** (1988) were not able to antagonize the TFMPP-cue by propranolol or mesulergine. Instead, propranolol, pindolol and mesulergine generalized to the TFMPP-stimulus, suggesting that the putative 5-HT1A antagonists may have agonistic properties on certain populations of 5-HT1B receptors. Our findings (Olivier **et al.**, 1987b; Olivier and Mos, 1988a) and those of Kruck **et al.** (1987) that propranolol exerts anti-aggressive effects may be related to a 5-HT1B agonistic character of propranolol.

Interestingly, Glennon **et al.** (1988) also suggested the involvement of a 5-HT1C mechanism in the stimulus properties of TFMPP. Because eltoprazine exerts 5-HT1C antagonistic activities and both TFMPP and RU24969 agonistic ones, this again supports our hypothesis that 5-HT1C-receptors are not involved in the modulation of aggressive behaviour.

Benzodiazepines

Benzodiazepines may exert both aggression enhancing and decreasing effects (cf. Miczek, 1987) and the literature covering these studies is quite confusing and sometimes,

certainly when regarding human data, of an anecdotical nature (for a review see Mos and Olivier, 1987; Mos **et al.**, 1987a; Rodgers and Waters, 1985). Benzodiazepines act via an action on BDZ-receptors which are closely linked to GABA-receptors and enhance GABAergic transmission in the CNS. This intringuingly complex GABA-BDZ receptor system is thought to be involved in many different behavioural processes, including aggression.

High doses of BDZ-agonists decrease aggression, possibly due to the muscle-relaxant properties of these drugs. At low doses either no effect or even enhancement of aggression has been reported (cf. Mos and Olivier, 1987, Mos **et al.**, 1987a; Miczek, 1987). In our laboratory we have done quite a number of studies using several benzodiazepine ligands, including agonists, antagonists and inverse agonists. Table V summarizes the results we obtained over the last 6 years (1982-1988). It appeared that several factors influence the modulatory action of benzodiazepines in agonistic behaviour.

First, it is clear that if pro-aggressive actions occur, they only do so at low doses. At higher doses effects wane and at still higher doses aggression is reduced, probably due to muscle relaxation and/or sedation (hypnosis); the latter activities have been described for all (partial) agonists (cf. Pieri, 1986).

A second factor is the baseline level of aggression. We found (Mos and Olivier, 1987) that in lactating female rats the pro-aggressive effects of a low dose of chlordiazepoxide were more pronounced in low than in high base-line levels of aggression. Although ceiling effects may play a role in highly aggressive females, the general level of aggression was such that increases were possible.

A third factor is the aggressive model used. In hypothalamically induced aggression, chlordiazepoxide had no pro-aggressive effects. Because the effects of drugs in this paradigm is tested at threshold levels, pro-aggressive effects

Table V Effects of benzodiazepine ligands on aggressive behaviour in mice and rats: summary of own results

Ligand	Species (gender)	Aggression paradigm	Doses (route) mg/kg	Effects on aggression	Experimental remarks	Reference
Chlordiazepoxide (BDZ-agonist)	mouse (♂)	isolation-induced aggression	5-15 (po)	↑ (low doses) ↓ (high doses)		Olivier and Van Dalen (1982) Olivier et al. (1986)
	rat (♂)	Social Interactions (♂)	2.5-5 (ip)	↑ (both doses)	Non-resident isolate vs group-housed intruder (S3-strain)	Olivier and Van Dalen (1982) Olivier et al. (1984; 1986)
	rat (♂)	Resident-Intruder (♂)	2.5-10 (ip)	↓	Residential male meets group-housed intruder (Wezob-strain)	Olivier et al. (1984; 1986)
	rat (♂)	Mini-colony (α and β-residents)		↑ (biphasic)	Pro-aggressive effects most pronounced in β-males (S3-strain)	Mos and Olivier (1987; 1988) Mos et al. (1987)
	rat (♂)	Hypothalamically induced aggression	5-20 (po)	– ↓ (high doses)	no effects on thresholds except at high doses (muscle relaxation)	Olivier et al. (1986) Mos and Olivier (1987) This chapter
	rat (♀)	" "	5-20 (po)	–	no effects of CDP whether light or heavy intruders were used	Mos and Olivier (1987)
	rat (♀)	Maternal aggression	5-20 (po)	↑ (low doses) – (high doses)	lactating females with pups	Olivier et al. (1985; 1986) Olivier and Mos (1986) Mos et al. (1987)
	rat (♀)	" "	5 (po)	↑ (heavy intr) – (light intr)	proaggressive effect dependent on the quality of the intruder	Mos and Olivier (1987a,b) This chapter
	rat (♂♀)	Play-fighting	1.25-10(ip)	↑ (low dose) – (high dose)	juvenile rats display play fighting	Mos and Olivier (1987)
	rat (♂♀) rat (♀)	Muricide " "	>10 (ip) 5 (po)	– ↑	Experienced killers Naive killers – first experience	Olivier et al. (1986) Mos and Olivier(1987a,b)
Diazepam (BDZ-agonist)	rat (♀)	Maternal aggression	0.3-5 (ip) 1.25-5 (po)	↑ (low doses) –↓ (high dose)	lactating females with pups	Mos and Olivier (1987) This chapter
Oxazepam (BDZ-agonist)	rat (♀)	Maternal aggression	1.25-20(ip)	↑ (low doses) – (high dose)	lactating females with pups	Mos and Olivier (1987) this chapter
	rat (♂)	Resident-Intruder	5-20 (po)	↑ (all doses)	Residential male meets group housed intruder (S3-strain)	This chapter
Alprazolam (BDZ-agonist)	rat (♀)	Maternal aggression	1.25-5 (po)	↑(at 1.25 mg/kg) – (at 2.5 mg/kg) ↓ (at 5 mg/kg)	lactating female with pups	This chapter Mos and Olivier (1989)
Ro 15-1788 (BDZ-antagonist)	rat (♀)	Maternal aggression	1.25-10(ip)	– (all doses)	" "	Mos and Olivier (1986; 1987a,b)
β-CCE (BDZ-inverse) agonist	rat (♀)	Maternal aggression	10-40 (ip)	↓ (highest dose)	" "	Mos and Olivier (1987) This chapter
Ro 15-1788 + Chlordiazepoxide	rat (♀)	Maternal aggression	1.25 and 10 (ip) + 5 (po)	no antagonism of aggression	" "	Mos and Olivier (1987a,b)
Ro 15-1788 + Oxazepam	rat (♀)	Maternal aggression	1.25 and 10 ip + 2.5 (ip)	no antagonism of aggression	" "	Unpublished results

can be measured in the set up and ceiling effects can be excluded. The nature of the evoked aggression and the environment in which it is evoked may give a clue to the understanding of why benzodiazepines do not exert pro-aggressive actions under all circumstances. During hypothalamic stimulation, which occurs in an environment which is familiair to the stimulated animal but which is certainly not its territory, aggression occurs in a quite unnateral way, by direct stimulation of brain structures which leads to sudden and unprovoked attack. This kind of behaviour is dissociated from its normal internal and external stimuli and restraints. Such data strongly suggest that benzodiazepines have no direct effect on aggression (via "aggressive" neural substrates) but interfere via indirect ways, e.g. via fear (anxiolytic effects may thus lead to enhancement of inhibited aggression).

Such a notion is supported by findings (Mos **et al.**, 1987a,b) that the quality of the opponent det-rmines whether pro-aggressive effects can be detected. Heavy intruders, capable of very adequate defense against attacks, evoke more aggression from lactating females under chlordiazepoxide treatment compared to light intruders (Mos **et al.**, 1987b). Interestingly, and supportive for the idea that benzodiazepines do not act directly on aggression, but have some indirect modulating effects, the aggression performed by BDZ-treated females is completely normal and adequate to defeat such heavy opponents. The latter notion has also been described in studies on prey-catching ferrets (Apfelbach, 1978). In this case chlordiazepoxide only facilitated prey-catching when a large prey was presented while it was without effect when the usual smaller sized prey was offered.

We further elaborated this indirect modulating effects of BDZ to the influence of experience. When benzodiazepines are given to experienced mouse-killing rats (Olivier **et al.**, 1987a,b) no effects were noted except at very high doses where sedation/muscle relaxation precluded the behaviour. In naive

rats, chlordiazepoxide dramatically increased the number of animals killing a mouse during the first test. When vehicle-treated animals had repetitive experience (3 successive tests) with mice and still did not kill, chlordiazepoxide was no longer effective. However, after 1 test chlordiazepoxide was still able to enhance the number of killers. Apparently, naive rats are normally inhibited (fear?) to kill mice and only a limited number of rats kill (a strain-dependent phenomenon). Benzodiazepines, at low doses facilitate this behaviour, presumably by reducing the inhibition (or reduction of a threshold). If however, the negative experience has lasted too long (3 tests or more) this inhibition cannot be overcome anymore by BDZ. Here again we find tentative evidence for the hypothesis of an indirect modulatory effect of BDZ on aggression; in situations of uncertainty (first confrontation in life with a mouse) BDZ may facilitate the behaviour.

That uncertainty (or fear) may play an important role in the pro-aggressive effects of BDZ, may be further illustrated by the social status-dependent effects of chlordiazepoxide in the mini-colony situation. In this situation chlordiazepoxide has its main pro-aggressive action on the subordinate male although on the dominant male it still has such an effect. No base-line dependent effects were present in this case and the most likely explanation of the differences between the α- and β-male, was that direct social restraints rather than established relationships lead to suppression of aggression by the β-male, but he latter remains fully capable no behave more aggressively. Again the indirect modulatory effects of benzodiazepines emerge, presumably acting by removing certain inhibitory influences like the presence of a rival. It should be noted that the dominant male is still subject to inhibitory influences (probably from the subordinate male) which becomes visible after BDZ-treatment. Again uncertainty may be an important factor determining the outcome of the behaviour.

Summarizing, BDZ agonists at low doses which have no sedating of muscle-relaxing effects, are capable of enhancing aggression towards threatening conspecifics or prey. Evidence is presented for an indirect, modulatory effect of BDZ in the expression of aggressive behaviour, evidenced by the influence of the qualities of the opponent, experimental environment, experience of the treated individual and its social status. Although rate-dependent phenomena may play a role in the pro-aggressive effects of BDZs, this certainly cannot account for the behaviour alone. The hypothesis is put forward that (fear-induced) uncertainty may play a key-role in the possible outcome of BDZ-treatment on aggression.

Uncertainty is part of modern key concepts of stress research, viz. predictability and controllability (cf. Wiepkema, 1987). Wiepkema (1987) proposes a regulatory model of the role of emotions in normal behaviour in which an individual organism tries to correct for differences between "Istwert" and "Sollwert". If an organism observes a difference between these two "values", it is motivated to reduce this difference and this steers a physiological and ethological programme which has been proven in earlier situations as adequate. Of course the organism has to monitor the effects of the programme and the sensations coupled to this monitoring are called emotions. Positive emotions arise when the expectations are fullfilled or even better, negative emotions may stop and correct the programme. The intensity of such emotions depends on the magnitude of the difference between the expectations and the real Umwelt changes and on the biological importance of the change of the Umwelt itself. Wiepkema postulates that emotions should play a dominant role in those behaviour programmes of which the outcome is not fixed; this may be represented by uncertainty. It is possible that at this stage of behavioural integration benzodiazepines may exert their action. Such a hypothesis fits with the data observed on the factors mediating the outcome of treatment with BDZs on aggressive behaviour.

MODULATION OF AGONISTIC BEHAVIOUR

A complicating factor in the hypothesis that BDZ exert their pro-aggressive action via a direct action on the BDZ-receptor in the CNS is that we were not able to antagonize this pro-aggressive effect by the relatively pure BDZ-antagonist Ro15-1788 (Mos **et al.**, 1987a,b). This certainly complicates an easy interpretation of such effects and makes pharmacological manipulation rather difficult. As some authors have reported that they are able to antagozine pro-aggressive effects of BDZs by BDZ-antagonists (Miczek, personal communication) it is possible that our results are chance findings.
The possilibity that the pro-aggressive actions of BDZ-agonists are not mediated via BDZ-receptors has to be further investigated before it can be dismissed.

Another factor contributing to a further unravelling of the mechanism behind the pro-aggressive actions of BDZ could be the divergence in the BDZ-receptors. Recently (Langer and Arbilla, 1988) a subdivision of BDZ-receptors in ω1 (BZ1), ω2 (BZ2) and ω3 (peripheral) subtypes has been proposed, because the old nomenclature and classification was purely based on the benzodiazepine chemical class and availability of selective antagonists. The presence of several non-benzodiazepines with high affinity for the central (BZ1/BZ2) and peripheral receptors, including imidazopyridines, triazolopyridines and β-carbolines suggest the availability of several interesting possibilities for functional subdivisions. Most benzodiazepines are non selective ligands for the central ω1 and ω2 receptors, whereas several non-benzodiazepines show selectivity for ω1 receptors (zolpidem, CGS9896 and CL218872). Besides peripherally, the ω2-subtype receptor is also present centrally and selective ligands for this subtype have been found (e.g. alpidem).
There is clearly evidence for different anatomical localizations of these 3 subtypes, the ω1-type occurring preferentially in the molecular layer of the cerebral cortex, the central pallidum and the substantia nigra (Niddam **et al.**,

1987), ω2 in the dentate gyrus and the caudate putamen (Niddam **et al.**, 1987), whereas ω3 occurs both peripherally and centrally in the olfactory bulb and spinal cord (Basile and Skolnick, 1986). Several of the therapeutic actions and the side effects of ligands to all these receptor types, may be associated with selective interactions with either one of these subtypes. Further work is needed to unravel the contribution of these different ω-receptors in the pro-aggressive action of benzodiazepines.

A final remark refers to the relation between BDZ and the serotonin system. We have completed a study in which we coupled the anti-aggressive effects of fluprazine, a weak serotonin-agonist, with the pro-aggressive effects of chlordiazepoxide (Olivier **et al.**, 1986). One pro-aggressive dose of CDP (5 mg/kg po.) was not able to shift the anti-aggressive dose-response curve of fluprazine. This suggested that the effects of fluprazine occurred more "downstream" in the CNS than that of benzodiazepine-agonists. Benzodiazepine-agonists inhibit or lower the level of uncertainty which on its turn lowers the level of inhibition on aggression, resulting in more aggression.

Activation of 5-HT1B receptors results in a direct inhibition of aggression after which BDZ-agonists cannot have anymore pro-aggressive effects. At present it is merely speculation to try to delineate the wiring of these "behavioural" programmes in the central nervous system. Promising developments are, however, that autoradiographical studies using high-affinity labeling of benzodiazepines revealed quite distinct CNS-localizations (Richards **et al.**, 1986), whereas autoradiographical studies with eltoprazine (Sijbesma **et al.**, 1988) identified also specific regions for 5-HT1B sites. These anatomical data enable local application of drugs into such areas to study whether direct pharmacological interventions may lead to new vistas on the neurobiology of agonistic behaviour.

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