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A NEW EXPERIMENTAL APPROACH FOR DETECTING EMOTIONAL AND MOTIVATIONAL CHANGES PRODUCED BY NEUROACTIVE COMPOUNDS IN RODENTS

V. CUOMO, M.A. DE SALVIA, C. LACOMBA, M. TATTOLI and R. CAGIANO

Istituto di Farmacologia, Università degli Studi, Bari, Italy

Summary. - *A new potential approach for detecting subtle changes of emotional and motivational states in rodents is represented by the analysis of ultrasonic vocalizations emitted in a variety of situations. The ultrasonic calls differ somewhat in their physical characteristics depending on the species and on the situation. The results of our studies on the effects of various neuroactive substances on ultrasonic emissions during neonatal life and during sexual behaviour are briefly described here together with what is known of the biological function of the calls.*

Riassunto (Nuovi approcci sperimentali per la valutazione di alterazioni emozionali e motivazionali prodotte nei roditori da sostanze neuroattive). - *L'analisi delle vocalizzazioni ultrasoniche emesse in varie situazioni sperimentali è un mezzo di indagine estremamente sensibile per il rilevamento di sottili alterazioni emozionali e motivazionali nei roditori. Le caratteristiche fisiche delle emissioni ultrasoniche differiscono in rapporto alla specie ed alla situazione sperimentale. Nella presente rassegna sono riportati i risultati di alcuni nostri studi sugli effetti di varie sostanze neuroattive sulla vocalizzazione ultrasonica in età neonatale e durante l'attività sessuale e sono descritte le principali funzioni biologiche di tali emissioni.*

Introduction

Our recent findings [1-10] suggest that ultrasonic vocalization in rodents could be considered as a sensitive indicator of subtle emotional and motivational changes produced by the administration of neuroactive substances. In particular, there is evidence that all myomorph rodent species thus far investigated emit ultrasonic vocalizations during social interactions.

These calls have a frequency above 20 kHz, the upper limit of human sensitivity, and are produced by both infants and adults.

It likely appears that ultrasounds play an important role in echo-location and intraspecific communication. Their importance has been established or implicated for such interrelated aspects of sociality such as parent-neonate interactions, aggressive and sexual behavior.

Moreover, ultrasounds are also emitted during exploration and in response to aversive stimuli.

Since rodents have the capability to communicate over a wide range of audible and ultrasonic frequencies, it is interesting to understand why ultrasonic frequencies appear to be widely used.

Many of the carnivorous birds and mammals, which prey on rodents, hunt largely at night and rely to a great extent upon hearing to locate prey [11].

Therefore, due to predatory pressures, rodents may have been evolutionarily selected to communicate ultrasonically. Many birds, for example, are unable to hear above 25 kHz [12].

Ultrasounds also have a high rate of attenuation with distance and are easily deflected by even very small objects such as blades of grass or twigs [13]. Ultrasounds are further attenuated by humidity and dust particles in the air [14].

Consequently, predators possessing high frequency hearing, could be less alerted at a distance by ultrasonic than by audible calls. Although ultrasounds do not carry well across distances, they have the property of being easily localized [15].

Localization by means of interaural intensity differences is most efficient with high frequency sounds while the short length and suddenness of ultrasonic pulses could provide localization cues on the basis of interaural differences in time of arrival. The ease with which rodent mothers can echo-locate calling pups empirically supports this contention. Thus,

ultrasonic cries provide the required highly directional signal over a short distance without communicating the location of the sender to a predator over a long distance.

In this report are briefly summarized our results obtained by using ultrasonic response as an indicator of changes in emotionality and motivation produced in rats by the administration of various neuroactive substances.

In particular, the effects of several compounds on ultrasonic emission during neonatal life and during sexual behaviour will be described.

Ultrasonic vocalization during neonatal life

Born in a relatively underdeveloped state, rat pups readily emit ultrasonic distress signals when removed from their nest [16-18] mainly in response to cold stress and/or tactile stimulation.

The calling of young mammals when separated from their nest is a near universal response although, in some species, the calls are ultrasonic and unnoticed by human observer.

The number of these emissions progressively decreases until when they develop homoiotermy.

These signals are used as cues to stimulate parental retrieval of lost infants, to elicit orientation toward the source of sound in lactating female rats [19], to reduce maternal aggression and rough handling of the pups and to promote nest building behaviour [20, 21].

As separation calling offers a stereotyped behaviour of obvious evolutionary importance, it is surprising that it has been the subject of relatively little psychopharmacological investigation.

Moreover, this response seems to be a sensitive index of the ontogeny of emotionality in rodents.

Effects of prenatal exposure to methyl mercury (MMC)

We recently demonstrated behavioural and neurochemical alterations in offspring of rats exposed to methyl mercury during gestation [22]. Infant ultrasonic vocalization was also affected by developmental exposure to methyl mercury [5].

The particular period of administration of MMC during pregnancy plays a critical role in determining changes of ultrasonic vocalization in neonate rats. In fact, the administration of a single dose of MMC (8 mg/kg) on the 15th day of gestation didn't produce any alteration in rat pup ultrasonic vocalization (unpublished data); on the contrary, its administration on day 8 of gestation produced marked changes of some parameters of these ultrasonic signals such as length and frequency. In particular, the duration of ultrasonic calls was significantly increased from day 4 up to day 8, while both maximum and minimum frequency values were shortened in animals exposed to this heavy metal.

Effects of prenatal exposure to methylazoxymethanol (MAM)

We have carried out experiments in order to investigate, in infant rats, the effects of prenatal administration of MAM on ultrasonic vocalization [4]. MAM, a potent alkylating agent, produces, when injected at the beginning of the 3rd week of pregnancy, a marked microencephaly as well as long-term cognitive impairments in the progeny of rats [23, 24].

The administration of a single dose of MAM (20 mg/kg) i.p. on day 13 of gestation produces a significant decrease in the duration of ultrasonic calls in both male and female pups from day 6 up to day 10 of life. These findings contribute to further characterize MAM-induced behavioural alterations that are considered to be a potentially useful animal model of congenital microencephaly with associated mental retardation.

Effects of prenatal and early postnatal treatment with dopamine (DA) receptor blocking agents

The ultrasonic vocalization of rat pups is differently altered by prenatal or postnatal administration of a dopamine-receptor blocking agent, like haloperidol.

The prenatal treatment with this neuroleptic agent produced only a slight decrease in the rate of ultrasonic vocalization in 4 day old male pups removed from their nest, whereas profound and long lasting changes in several parameters of ultrasonic vocalization were found in pups exposed to haloperidol during early postnatal life [1, 8].

In particular, the neonatal administration of this neuroleptic agent produced a significant decrease in the rate as well as in maximum and minimum frequency of calling, whereas a significant increase in the duration of calls was found in pups exposed to haloperidol.

The different results obtained with prenatal or early postnatal exposure to haloperidol confirm that the particular period of administration of dopamine receptor blocking agents plays a critical role in producing short- and long-term behavioural changes in rats. The marked alterations in ultrasonic emission elicited by the neonatal exposure to haloperidol could be due to the interaction of this neuroleptic with the development of the dopaminergic neurotransmission during the most vulnerable period for the functional maturation of this neurotransmitter system in the rat.

To evaluate the role of DA1- and DA2-receptor populations in the ultrasonic emission during early postnatal life, we also investigated the effects of a prolonged postnatal treatment with selective DA1 (SCH 23390)- and DA2 (sulpiride)-receptor blocking agents on ultrasonic calling in rat pups [3].

The results of our studies showed that the prolonged postnatal administration of SCH 23390 and sulpiride significantly altered the ultrasonic vocaliz-

ation in rat pups. However, a comparative evaluation of the results indicates that ultrasonic calling was differently affected by SCH 23390 and sulpiride, respectively. In particular, the neonatal exposure to SCH 23390 produced a significant increase in the duration of calls and a significant decrease in their minimum and maximum frequency values, whereas sulpiride did not modify these ultrasonic parameters. In this regard, the effects of SCH 23390 on the length, as well as on the frequency of calls, were similar to those elicited by haloperidol.

On the other hand, unlike SCH 23390, sulpiride significantly reduced the rate of calling and the sound pressure level of ultrasounds. The effects of sulpiride on the rate of emission paralleled those produced by the neonatal exposure to haloperidol. These data indicate that the different ultrasonic alterations produced by the early postnatal administration of SCH 23390 and sulpiride could be due to an impaired maturation of DA1- and DA2-receptor populations, respectively. Moreover, it would seem that these two types of DA receptors play a distinct role in the ultrasonic vocalization of rat pups.

Effects of developmental exposure to opiates

Since pup ultrasonic calls are considered to be «distress calls» and evidence is accumulating that endogenous brain opioid systems seem to be involved in the modulation of separation-induced distress vocalization in several animal species [25-27], we have also studied the consequences of early postnatal exposure to morphine on ultrasonic vocalization in rat pups.

Moreover, since it has been demonstrated that beta-casomorphins (beta-CMS), which are opioid peptides derived from enzymatic digestion of milk protein (beta-casein), significantly affect separation-induced distress vocalization in young chicks [28], the effects of their prolonged administration on ultrasonic calling in rat pups were also investigated. Finally, the effects of naloxone, an opioid antagonist, were evaluated.

The results of this investigation [9] showed that only morphine-exposed pups exhibited significant changes in ultrasonic emission. In particular, a significant decrease in the rate of calling was found in morphine-treated animals from day 4 up to day 12 after birth. Moreover, morphine significantly increased the duration of calls from the 4th to the 10th day of life.

Finally, a significant reduction of the sound pressure level as well as of the range of frequency from day 6 up to day 12 was found in morphine-exposed pups. Conversely, neither β-CMS nor naloxone significantly affected ultrasonic emission. Finally, the changes in ultrasonic vocalization of rat pups elicited by morphine give further support to previous findings suggesting that this behavioural parameter could be valuable as bioassay in developmental pharmacology and toxicology.

Ultrasonic vocalization during sexual behaviour

Ultrasonic calls have been detected also during sexual behaviour in all species of murid rodents that have been adequately studied. The calls are commonly emitted either before or after copulation. Such sexual calling is often characterized by rapid and marked changes in frequency within single calls. For example, the pre-ejaculatory calls of rats consist of a single frequency component that shows rapid and marked frequency modulations, each lasting a few milliseconds. These pre-ejaculatory calls appear to be emitted by the male and to reflect levels of sexual motivation [33].

They are commonly called «50 kHz calls» because the greatest part of their energy is emitted at 50 kHz. Moreover, these precopulatory calls stimulate proceptive behaviour (ear wiggling and darting) as well as receptive behaviour (lordosis) in female rats [34-36].

Furthermore, male rats also emit ultrasonic calls (22 kHz) after ejaculation. Each 22 kHz call has a very long duration (1 to 3 s) and can also reach an intensity peak of 80 dB sound pressure level [37].

Post-ejaculatory vocalizations (PEV) show a frequency range of 20-30 kHz [38, 39].

A sleep-like EEG pattern and little movement are displayed by male rats during PEV [40].

These emissions are commonly called «22 kHz calls» because the greatest part of their energy is emitted at 22 kHz. They are related to the absolute refractory period during which the male is incapable of renewed sexual activity. Their communicative function is not yet established, but it has been suggested to function as a territorial announcement [41].

It has also been proposed that 22 kHz calls serve to discourage other males from mating with the same female and/or to keep the female at a distance during total post-ejaculatory refractory period [39, 42] although this latter function has been disputed [43].

Effects of the administration of p-chlorophenylalanine (pCPA)

We have carried out experiments on the effects of pCPA, an inhibitor of serotonin synthesis which stimulates sexual activity in rodents [44], on ultrasonic vocalization of male rats [10].

The results obtained in this experiment showed that the prolonged administration of pCPA, at a dose level producing a 90% depletion of serotonin content in the cerebral cortex of male rats, produced significant changes either on ultrasonic vocalization during sexual behaviour or on copulatory behaviour.

In particular, the administration of pCPA significantly reduced the latency of emission of the first pre-copulatory 50 kHz ultrasound: to our knowledge, this is the first demonstration that the administration of pCPA will shorten the time of appearance of an ultrasonic response (50 kHz calls) which is a sensitive index of sexual motivation in male rats.

Furthermore, pCPA markedly increased the duration of the period of 22 kHz PEV and decreased the latency of its appearance.

These results are in agreement with the data on copulatory behaviour; in fact, pCPA administration significantly reduced the latency of the first mount or intromission and the latency of ejaculation.

Effects of prenatal exposure to dopamine receptor blocking agents

Since there is evidence that dopamine plays an important role in the regulation of sexual behaviour in rats [45-47] and that dopaminergic mechanisms are suspected to underly sexual dysfunctions produced by the administration of some psychotropic drugs such as haloperidol [48-50] , it was of interest to investigate the effects of prenatal treatment with haloperidol on 22 kHz post-ejaculatory vocalization (PEV) in adult rats.

The results of this study show that, even though the prenatal treatment with haloperidol doesn't affect the typical parameters of male rat sexual activity, significant alterations in 22 kHz PEV of haloperidol-treated males have been found. In fact, male rats prenatally treated with haloperidol exhibited a significant increase both in duration and intensity of 22 kHz PEV with respect to controls [8].

Effects of prenatal exposure to methyl mercury (MMC)

In a collaborative study on testing strategies in behavioral teratology, we studied the effects of prenatal administration of MMC, at different dose levels, on male ultrasonic vocalization during sexual behaviour [6].

The results of this preliminary study did not reveal any significant difference between treated and untreated rats.

Conclusions

The data reported in this brief review suggest that ultrasonic vocalization in rats may represent a new potential approach in developmental pharmacology and toxicology. In particular, the ultrasonic response during neonatal life, which is an indicator of the ontogeny of emotionality, is so predictable and reproducible that it could be considered as one of the parameters to be scored in a toxicological test battery.

Furthermore, our data suggest that ultrasonic calling during sexual encounters could serve as an accurate tool for detecting alterations in sexual motivation resulting from treatment with some pharmacological agents during development.

In particular, ultrasonic vocalization may represent a sensitive indicator of subtle emotional and motivational changes produced in rats by perinatal administration of drugs at dose levels below those associated with overt signs of neurotoxicity.

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