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KINDLING INCREASES AVERSION TO SACCHARIN IN TASTE AVERSION LEARNING

L. LÓPEZ-VELÁZQUEZ, E. AGUIRRE AND R. G. PAREDES*

Instituto de Neurobiología, Universidad Nacional Autónoma de México, Apartado Postal 1-1141, Querétaro, Qro. C.P. 76001, Mexico

Abstract—Kindling is a model in which an initially subconvulsive electrical stimulation of certain brain areas eventually develops a generalized seizure that produces behavioral and long term neuronal changes. In the present study we evaluated if kindling can modify conditioning taste aversion (CTA). In this paradigm animals acquire aversion to saccharin when it is presented as the conditioned stimulus (CS) followed by an injection of lithium chloride (LiCl) that induces a gastric irritation as the unconditioned stimulus (US). Male Wistar rats were implanted with bipolar electrodes aimed at the right amygdala (AMG) or at the right insular cortex (IC). The animals were stimulated daily until they reached stages 2–4 (intermediate) or until kindling was fully established (three consecutive stage 5 seizures). At least two weeks after kindling stimulation had ceased the animals were deprived of water for 24 h and given 10-min drinking sessions twice a day for 4 days. On day 5 (morning session) tap water was replaced by saccharin solution (0.1%), 20 min later the animals were injected with LiCl (7.5 ml/kg i.p., 0.2 M) to induce gastric malaise or taste aversion. After three more days of baseline consumption, water was substituted by a fresh 0.1% saccharin solution to test the aversion. AMG-kindling delayed the extinction of CTA. Animals with kindling in the IC had a higher retention than the sham kindling group; that is, they drank significantly less saccharin solution than the other groups. The results of the present experiment show that local modification of brain function induced by kindling stimulation can prolong the aversive effects of CTA.

Key words: kindling, conditioned taste aversion, amygdala, insular cortex, learning and memory.

Kindling is a model in which an electrical subconvulsive stimulation is administered daily in certain brain areas and eventually develops generalized seizures that produce behavioral and long term neuronal changes (Goddard et al., 1969; Goddard and Douglas, 1975). Several studies have demonstrated that kindling can induce behavioral changes. Previously we showed that male rats that did not display sexual behavior, despite being tested on repeated occasions with sexually receptive females, started mating after kindling was established in the medial preoptic area (Paredes et al., 1990). These males continued to display sexual behavior even 8 months after kindling stimulation had ceased (Portillo et al., 2003). These studies clearly show that kindling produces long term behavioral changes probably associated with the specific brain area stimulated. Other groups have also reported behavioral changes after kindling including modifications in anxiety (Adamec and Young, 2000) and maternal behavior (Morgan et al., 1999). Several studies have asses the effects of kindling like stimulation upon learning (see (Hannesson and Corcoran, 2000) for a review). In general, these studies have described alterations in learning and memory (Gilbert et al., 2000; Hannesson et al., 2001, 2005; Anisman and Mclntyre, 2002). However, the effects of kindling upon learning could be different depending upon the interval between the last seizure and behavioral training. For example it has been shown that when longer periods (7 weeks) are used between kindling stimulation and the behavioral task no impairment of cognitive function is observed (Cammisuli et al., 1997; Leung and Shen, 2006).

The conditioned taste aversion (CTA) paradigm consists of the presentation of a novel flavor (usually saccharin) followed by gastric malaise (usually induced by injection of lithium chloride; LiCl) that produces in the animal a subsequent aversion to the taste. This is a suitable model of learning and memory to study neural plasticity. It requires only one presentation or pairing of the stimuli to establish conditioning. Another important aspect is that the brain areas that participate in CTA have been clearly identified. Several studies have demonstrated the importance of the insular cortex (IC) in the acquisition and storage of CTA (Bermúdez-Rattoni and McGaugh, 1991; Bermúdez-Rattoni and Yamamoto, 1998; Gutiérrez et al., 2003). Different groups have shown that the basolateral amygdala (BLA) plays a role in the formation of CTA (Yasoshima et al., 1995; Miranda et al., 2002) whereas the central amygdala (cAMG) is involved in the recognition of the meaning of the conditioned stimulus (CS) (Yasoshima et al., 1995). There is also evidence indicating that the cAMG is important in the acquisition of CTA (Bahar et al., 2003). Likewise, Lamprecht et al. (1997) demonstrated that inhibition of protein synthesis and blockade of the transcription factor cAMP-response element-binding protein (CREB) in cAMG blocked consolidation of CTA memory.

Most of the studies that have assessed the effects of kindling stimulation upon CTA have reported an impairment of the task. In these studies the stimulation was given immediately after training or between the CS and the unconditioned stimulus (US) evaluating only short term ef-

*Corresponding author. Tel: +52-442-2381060; fax: +52-422-2381046.
E-mail address: rparedes@servidor.unam.mx (R. Paredes).

Abbreviations: AD, afterdischarge; AMG, amygdala; AP, anteroposterior; BLA, basolateral amygdala; cAMG, central amygdala; CREB, cAMP-response element-binding protein; CS, conditioned stimulus; CTA, conditioned taste aversion; DV, dorsoventral; IC, insular cortex; LiCl, lithium chloride; US, unconditioned stimulus.

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fects of kindling stimulation associated with seizure induced alterations (Mikulka and Freeman, 1984; Peele and Gilbert, 1992). As well, these studies have evaluated only one day of the extinction curve. If kindling stimulation does indeed produce long term brain function modification, one way to examine these changes would be to induce kindling and assess behavioral changes several days later including the extinction curve. This would give more emphasis to the long term changes induced by kindling stimulation. The aim of the present study was to evaluate if long term effects of kindling (19 days after the last stimulation) could modify acquisition or extinction of CTA. For that purpose different groups of animals were stimulated daily until they reach stages 2–4 (intermediate) or until kindling was fully established (three consecutive stage 5 seizures) in either the cAMG or IC. Sham-stimulated animals in the cAMG and IC were also included. Two weeks after kindling was established animals were trained for CTA.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats from a local colony, weighing 250–300 g, were maintained in single cages with an *ad libitum* feeding schedule under a 12/-h light/dark cycle; the temperature was kept within the range of 19–21 °C. All efforts were made to minimize the number of animals used and their suffering during experimental procedures according to the Reglamento de la Ley General de Salud en Materia de Investigación para Salud of the Mexican Health Ministry and the Institute Animal Care Committee that follows National Institutes of Health (NIH, USA) guidelines.

Surgery

The rats were deeply anesthetized with a mixture of ketamine (95 mg/kg) and xylazine (12 mg/kg) injected intraperitoneally. They were surgically implanted with a bipolar stainless steel insulated electrode at the right cAMG, or the right IC. The following coordinates based on the atlas of Paxinos and Watson (1987) were used: cAMG: anteroposterior (AP) −2.5 mm from bregma; 4.25 mm lateral to the midline; dorsoventral (DV), −8.0 mm from dura madre. IC: AP+1.2 mm from bregma; 5.5 mm lateral to the midline, DV, −5.5 mm from dura mater. Three stainless steel anchor screws were placed in the skull. The rats were returned to their home cage for a 1 week recovery period.

Procedure

After recovery, kindling procedure was initiated. Rats were individually transferred to the kindling room and were attached to a cable connected to a custom-built constant-current generator. On day 1 the afterdischarge (AD) threshold was determined for each rat. The stimulation consisted of a 1-s train of 60-Hz biphasic square-wave pulses of 1 ms duration. Initiating at 100 μA the stimuli were given in 50 μA increments until an AD was observed. The duration of the AD was determined from electroencephalographic recordings. The animals were subsequently stimulated twice a day at this threshold. The behavioral progression of kindling induced seizures was scored according to Racine's standard classification (Racine, 1972): stage 1, mouth and facial movements; stage 2, stage 1 and head nodding; stage 3, stage 2 and unilateral forelimb clonus followed by contralateral clonus; stage 4, stage 3 and rearing; stage 5, stage 4 and loss of postural control and falling. The animals were stimulated until they reach stages 2–4 (intermediate) or until kindling was fully established (three consecutive stage 5 seizures). Sham-stimulated animals were treated identically, but without any electrical stimulation. In this way a total of six groups were formed: sham, intermediate (stages 2–4) and fully kindled animals in the IC, and similar groups in the amygdala (AMG). At least two weeks after kindling was established CTA was initiated. The animals were deprived of water for 24 h and given 10 min drinking twice a day for 4 days. On session 1 (acquisition day, morning of day 5) tap water was replaced by saccharin solution (0.1%), 20 min later the animals were injected with LiCl (7.5 ml/kg i.p., 0.2 M) to induce gastric malaise or taste aversion. In sessions 2, 3 and 4 water was given in the morning and afternoon sessions to obtain baseline consumption. In the morning of session 5 the water given in the morning was substituted by a freshly 0.1% saccharin solution to test the aversion (session 5) and extinction (sessions 6–9). The pairing between the CS (saccharin) and the US (LiCL) occurred at least 19 days after the last kindling seizure while testing (presentation of the CS) was done at least 23 days after.

Histology

At the end of the experiment the rats were killed and perfused using standard procedures. The brains were removed from the cranium and were placed in 30% sucrose/PBS solution for cryoprotection. Brains were frozen and cut in the coronal plane at 35 μm sections. Later they were mounted and stained with Cresyl Violet for histological verification of electrode placements.

Statistical analysis

The number of stimuli and the number of AD to produce stage 5 were analyzed with a *t*-test. AD duration was analyzed by a 2 (group)×9 (sessions) ANOVA for repeated measures of the session factor. These parameters were analyzed only for the two groups (cAMG and IC) stimulated until stage 5. Effects of kindling on CTA were analyzed by a 2 (place of implantation: cAMG and IC)×3 (kindling stage: sham, intermediate kindling and full kindling)×9 (CTA sessions) ANOVA for repeated measures on the session factor. In case of significant effects, comparisons were done by Fisher post hoc test.

RESULTS

Kindling

No significant differences in the number of AD needed to reach stage 5 were found between cAMG and IC fully kindled animals (cAMG 8.8±3.32 vs. IC 7.5±1.53; $t_{(17)}=0.36$). Significant effects were observed in the duration of the AD [group ($F_{(1,\ 18)}=48.69$, $P<0.0001$), number of AD ($F_{(9,\ 18)}=22.79$, $P<0.0001$) and interaction ($F_{(9,\ 18)}=9.97$, $P<0.0001$)]. As can be see in Fig. 1, the AD duration in the cAMG group was significantly longer than that seen in the group stimulated in the IC.

Kindling and CTA

The ANOVA revealed a significant effect of kindling stage ($F_{(2,\ 51)}=8.22$, $P=0.01$), session ($F_{(8,\ 51)}=147.70$, $P<0.01$) and interaction ($F_{(16,\ 51)}=11.57$, $P=0.034$). The post hoc test showed significant differences between the sham group and animals with full kindling in the cAMG (AMG-kindling) during sessions 6, 7, 8 and 9 ($P<0.01$). The animals kindling in the cAMG drank significantly less saccharin than animals with sham kindling, prolonging the extinction of CTA. Rats with intermediate kindling (stages