6-21-1990

Blocking of Acquisition But Not Expression of Conditioned Fear-Potentiated Startle by NMDA Antagonists in the Amygdala

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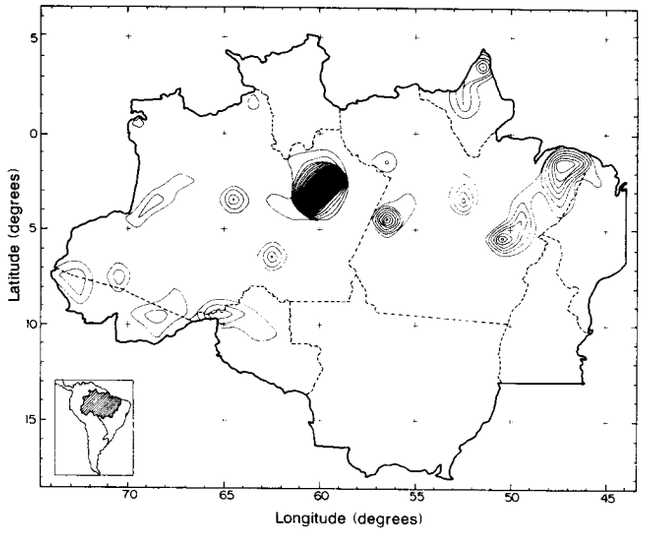

FIG. 4 Same data as shown in Fig. 3 treated by putting to zero all degree-grid squares where collections were known to be primarily from areas unlikely to have been forested during a drier Pleistocene, that is, savanna areas, transition forest, high forest close to savanna or transition forest (such as at Jari, Aripuanã and Obidos), white sand areas, sandstone or canga formation and floodplain or low-lying *terra firme* areas downstream of Manaus, probably inundated during previous Pleistocene interglacials.

centre and not suspected of being an artefact. Of the three refugia on international borders, the East Guiana refuge is heavily collected in its Brazilian portion. The other two are based largely on collections from outside Brazil.

Our analysis does not weaken the arguments for endemism centres and refugia based on the distribution of birds[7,8], butterflies[9,10] and lizards[11], although in the case of birds, at least, the data are subject to alternative interpretations[12]. With the collecting density map presented here it may now be possible for phytogeographers and conservation planners interested in identifying endemism centres to correct for the strong bias in the botanical sample. Centres of diversity determined by superimposing the known ranges of hundreds of species should incorporate similar corrections. As the phytogeographical data base is highly biased towards collection centres, conservation planners aiming to preserve unique plant communities should give greater weight to more equitably distributed co-variate data. These include maps of climate, geology and soil, as well as remote sensing of geomorphology and vegetation physionomy coupled with rapid standardized field inventories of forest diversity, structure and composition. Our map clearly indicates the most critical area for future botanical collecting efforts: deforestation in the Brazilian Amazon is concentrated in southern Pará and northern Mato Grosso, a large, botanically unexplored area. □

Received 21 December 1989; accepted 2 April 1990.

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Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala

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RECEPTORS for *N*-methyl-D-aspartate (NMDA) seem to have a critical role in synaptic plasticity[1–4]. NMDA antagonists (such as AP5) prevent induction of long-term potentiation[5,6], an activity-dependent enhancement of synaptic efficacy mediated by neural mechanisms that might also underlie learning and memory. They also attenuate memory formation in several behavioural tasks[7–17]; there are few data, however, implicating an NMDA-sensitive measure of conditioning based on local infusion of antagonists into a brain area tightly coupled to the behavioural response used to assess conditioning. We now show that NMDA antagonists infused into the amygdala block the acquisition, but not the expression, of fear conditioning measured with a behavioural assay mediated by a defined neural circuit (fear-potentiation of the acoustic startle reflex). This effect showed anatomical and pharmacological specificity, and was not attributable to reduced salience of the stimuli of light or shock used in training. The data indicate that an NMDA-dependent process in the amygdala subserves associative fear conditioning.

The role of NMDA receptors in the amygdala in associative fear conditioning was assessed using fear-potentiation of acoustic startle, a simple reflex mediated by a defined neural pathway contained in the brainstem and spinal cord[18]. The central nucleus of the amygdala projects directly to one of the brainstem nuclei critical for startle[19] and lesions of this amygdaloid nucleus, or interruption of the pathway connecting the amygdala to the startle circuit, block the ability of conditioned or unconditioned fear stimuli to elevate startle[20,21]. Conversely, weak electrical stimulation of the amygdala markedly increases startle amplitude[22]. The lateral and basolateral amygdaloid nuclei, which project directly to the central nucleus, contain high densities of NMDA receptors[23] and lesions of these nuclei markedly attenuate the acquisition of fear-potentiated startle (C.B.S. and M.D., unpublished observations). If an NMDA-dependent process in the amygdala is involved in associative fear conditioning, then such conditioning should be sensitive to manipulations of local NMDA receptor function.

To test this, 132 rats were implanted with bilateral cannulae aimed at the basolateral amygdaloid nuclei. Thirteen control animals were implanted with cannulae aimed at the interpositus nuclei of the cerebellum, an area involved in classical conditioning of motor responses, but not in fear conditioning[24]. One week after surgery, animals were bilaterally infused on two successive days with 0.5 μl of one of the following: vehicle (artificial cerebrospinal fluid (ACSF) or 0.1 M phosphate buffer, $n = 23$); DL-2-amino-5-phosphonopentanoic acid (AP5; 6.25 ($n = 5$), 12.5 ($n = 9$), 25 ($n = 13$) or 50 nmol ($n = 5$), DL-2-amino-7-phosphonoheptanoic acid (AP7, 50 nmol ($n = 6$); ACSF ($n = 5$) or propranolol (40 nmol ($n = 7$); ACSF ($n = 7$)). Solutions were adjusted to pH 7.0. Five minutes later, animals were presented with 10 light–footshock pairings at 4–6-min intervals. Conditioning was assessed one week later by comparing startle elicited by a noise burst presented alone, with startle elicited by a noise burst in the presence of the light previously paired with footshock. Magnitude of conditioned fear was defined as the increase in startle amplitude on the light–noise versus noise-alone trials.

Infusion of AP5 or AP7 into the amygdala blocked the acquisition of fear-potentiated startle relative to vehicle controls (Fig. 1*a*, *b*). But, the β-adrenergic antagonist propranolol, which attenuates one-trial inhibitory avoidance conditioning when infused into the amygdala after training[25] and has powerful local anaesthetic effects[26], did not attenuate the acquisition of fear-potentiation (Fig. 1*c*). No motor impairment (such as catalepsy or ataxia) was observed after local infusion of any of these doses of AP5 into the amygdala. Bilateral local AP5 (100 nmol) administration into the interpositus nucleus of the cerebellum before training ($n = 8$) did not affect fear-potentiation of startle, even at a dose eight times that which blocked conditioning in the amygdala (Fig. 2*a*). In addition, animals infused with AP5 5 days after training, but 1 week before testing ($n = 8$), did not differ at testing from vehicle controls ($n = 5$; Fig. 2*b*), indicating that the observed block of acquisition by AP5 did not result from any permanent damage to the amygdala that might have developed over the 1-week infusion-test interval. Moreover, animals with AP5 (50 nmol) infused into the amygdala reacted as vigorously as controls to the 0.6 mA footshock used in training (Fig. 2*c*). This footshock-induced increase in activity has previously been shown to be markedly reduced by lesions of the amygdala[21] or systemic injection of morphine (J. M. Hitchcock and M. Davis, unpublished observations). This suggests that AP5 does not block the acquisiton of fear conditioning by producing analgesia or by decreasing the ability of the footshock to activate the amygdala.

To test whether a smaller amount of drug would block conditioning, a shorter training session was used consisting of five light–shock pairings presented at a 2-min interval. Under these conditions, infusion of AP5 5 min before training blocked the acquisition of potentiated startle in a dose-dependent manner and significantly attenuated fear conditioning at one-fourth of the dose required to block conditioning using the original, longer training procedure (Fig. 3*a*; ACSF ($n = 5$); AP5 1.6 ($n = 5$); 3.12 ($n = 6$); 6.25 ($n = 6$); or 12.5 nmol ($n = 6$)). Importantly, however, even the highest effective dose of AP5 did not block the expression of fear-potentiated startle in animals trained in the absence of the drug (Fig. 3*b*; ACSF ($n = 5$); AP5 12.5 nmol ($n = 6$)). This strongly suggests that local infusion of AP5 into the amygdala does not prevent synaptic transmission of visual information to the amygdala.

Histological examination of cannula placements in a subset of animals ($n = 20$) suggests that the compounds used in this

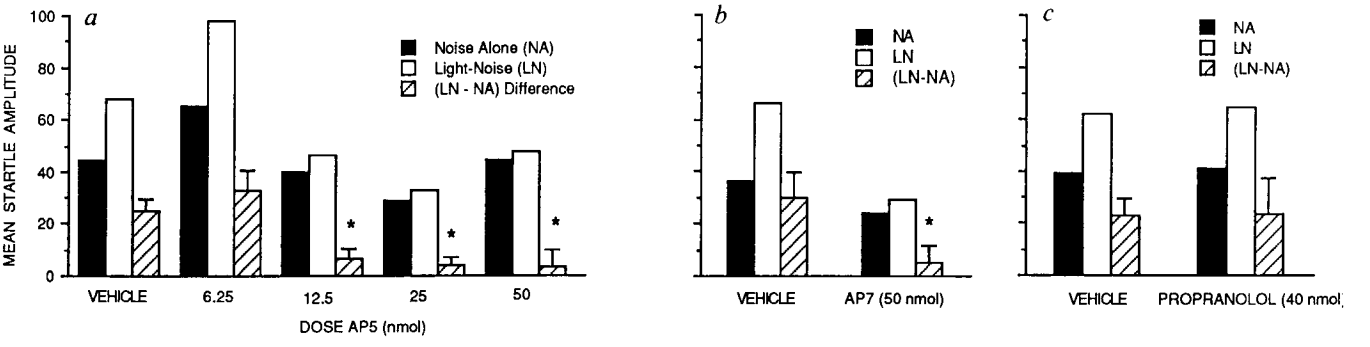

FIG. 1 NMDA-specific antagonists infused into the basolateral nuclei of the amygdala immediately before light–shock training sessions blocked the acquisition of conditioned fear measured with potentiated startle tested 1 week later. *a*, AP5 caused a dose-dependent decrease in conditioned fear-potentiation of startle (F_{linear} (1,50) = 14.75, $P < 0.001$), with significant attenuation compared with vehicle at doses of ≥12.5 nmol ($P < 0.05$, Newman-Keuls). *b*, AP7 also blocked fear-potentiated startle ($t(9) = 2.23$, $P < 0.05$). But rats receiving bilateral infusion of propranolol *c*; (40 nmol) into the amygdala at training showed significant fear-potentiation of startle, ($t(6) = 2.92$, $P < 0.03$) and did not differ from vehicle control animals at testing ($t(12) = 1.32$, not significant). The apparatus and potentiated startle training and testing procedures have been described previously[28,29]. The standard potentiated startle paradigm require two 45-min training sessions.

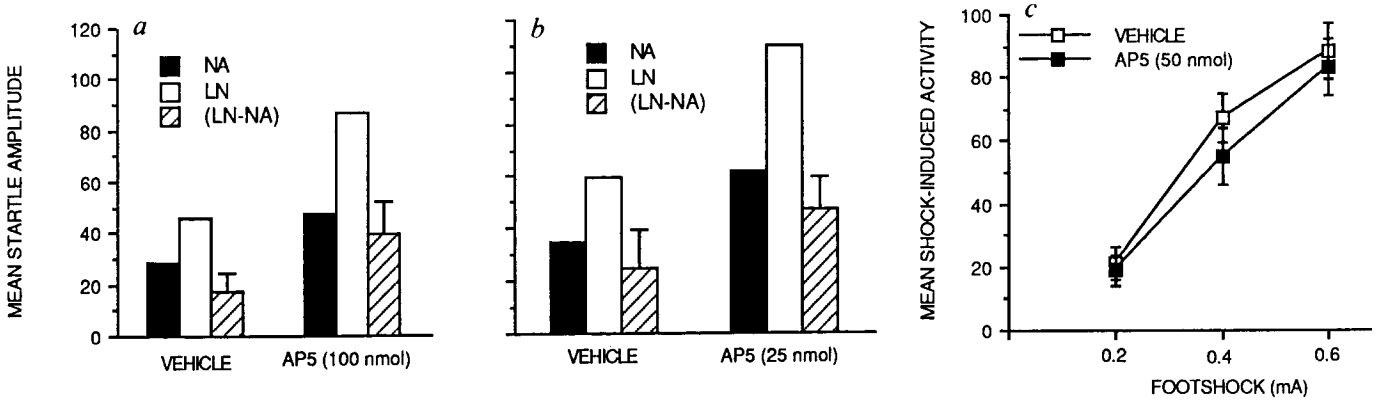

FIG. 2 *a*, Infusion of a high dose of AP5 (100 nmol) into the cerebellar interpositus nuclei at training did not block or attenuate fear-potentiation of acoustic startle ($t(12) = 1.43$, not significant). *b*, Startle amplitude at testing was not attenuated as a result of AP5 (25 nmol) infusion into the amygdala, on two successive days, 5 days after standard potentiated startle training ($t(11) = 1.21$, not significant). As in the previous experiments, the infusion-testing interval was 1 week. *c*, Reactivity to footshock was not different for animals given AP5 (50 nmol ($n = 10$)) or vehicle ($n = 10$) into the amygdala ($F(1, 18) = 0.84$, not significant). During shock-activity testing, rats received ten 500 ms shocks at 1-s intervals at each of the following intensities: 0.2, 0.4 and 0.6 mA, presented in ascending order. Activity was assessed by cage displacement for the 500 ms period after shock onset.

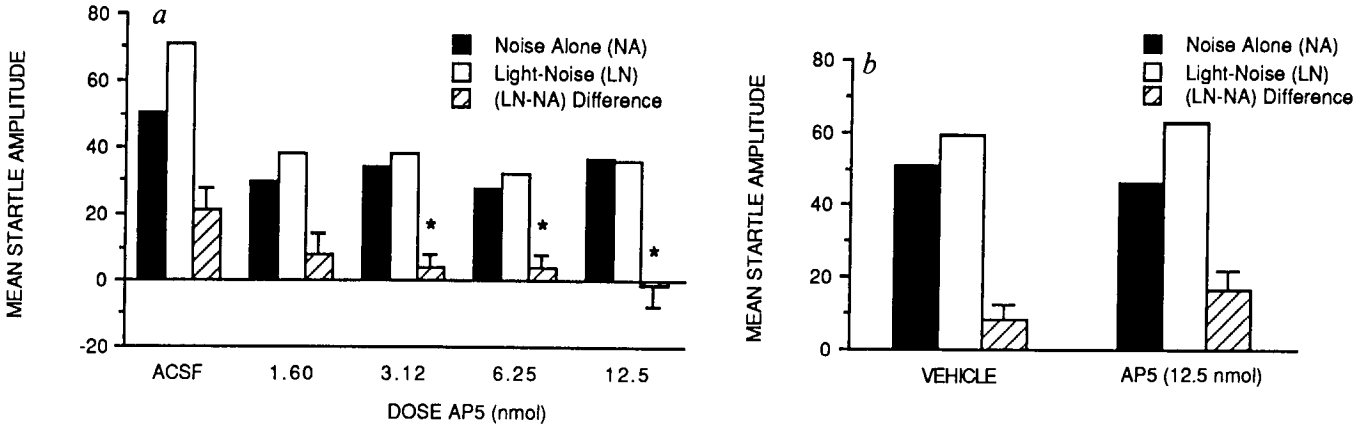

FIG. 3 *a*, Infusion of AP5 into the amygdala immediately before a shorter training procedure (a 15-min session consisting of five light (conditioned stimulus)-footshock (unconditioned stimulus) pairings, with a 2-min interstimulus interval) dose-dependently blocked acquisition of conditioned fear (F_{linear} (1, 23) = 7.43, $P < 0.01$), and significantly reduced potentiated startle at doses of 3.12 nmol and greater. *b*, The highest effective dose of AP5 (12.5 nmol) used to block acquisition of conditioned fear in the short training procedure did not attenuate the expression of fear-potentiated startle, compared with vehicle controls, when infused into the amygdala immediately before testing. Hence the overall difference between the light-noise and noise-alone trials was statistically significant ($F(1, 9) = 11.97$, $P < 0.01$) but there was no group by trial-type interaction ($F(1, 9) = 1.18$, not significant) and significant potentiated startle occurred in the AP5 group ($t(5) = 2.91$, $P < 0.03$).

study attenuated potentiated startle through actions on a substrate ≤1.0 mm from the basolateral nucleus. Therefore, two rats with one or both cannula tips located beyond that distance did not show a decrease in fear conditioning, even when normally effective doses were infused.

The finding that NMDA receptor antagonists infused into the amygdala blocked fear-potentiated startle indicates that an NMDA-mediated process at that level is critical for the acquisition of conditioned fear. The many demonstrations that NMDA antagonists also block induction of long-term potentiation (LTP), and the finding in intracellular studies that LTP occurs in the amygdala[27], suggest that an NMDA-dependent form of LTP in the amygdala might underlie fear conditioning.

Investigations of the physiological and biochemical mechanisms of learning and memory have mainly made use of *in vitro* preparations. The results of the present study, suggesting the existence of an NMDA-dependent process in the amygdala subserving fear conditioning, show that it may now be possible to use a behavioural measure, with a defined neural circuit tightly coupled to the possible site of plasticity, to evaluate the role of these mechanisms *in vivo*. □

Received 22 October 1989; accepted 6 April 1990.

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ACKNOWLEDGEMENTS. We thank Lee Schlesinger for help in training animals, K. C. Liang for comments and for collection of propranolol data, and J. B. Rosen, and T. H. Brown and workers in his laboratory for comments on the manuscript. This research was supported by the NIH, NIMH and AFOSR.

Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation

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IN both vertebrates and invertebrates, long-term memory differs from short-term in requiring protein synthesis during training[1,2]. Studies of the gill and siphon withdrawal reflex in *Aplysia* indicate that similar requirements can be demonstrated at the level of sensory and motor neurons which may participate in memory storage. A single application of serotonin[3], a transmitter that mediates sensitization, to individual sensory and motor cells in dissociated cell cultures leads to enhanced transmitter release from the sensory neurons that is independent of new macromolecular synthesis. Five applications of serotonin cause a long-term enhancement, lasting one or more days, which requires translation and transcription[2,3]. Prolonged application or intracellular injection into the sensory neuron of cyclic AMP, a second messenger for the action of serotonin, also produce long-term increases in synaptic strength[4,5], suggesting that some of the gene products important for long-term facilitation are cAMP-inducible. In eukaryotic cells, most cAMP-inducible genes so far studied are activated by the cAMP-dependent protein kinase (A kinase), which

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