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Fear conditioning performance and NMDA receptor subtypes: NR2A differential expression in the striatum

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Abstract

While considerable evidence implicates NMDA receptors in the hippocampus in contextual fear conditioning, the role of other brain regions is less well understood. To further investigate this issue, rats were subjected to a contextual fear conditioning task and then classified as high or low responders according to performance. Density of NMDA receptors was evaluated using [^{3}H]MK-801 autoradiography in 52 brain areas and expression of NR2A and NR2B subunits was studied with in situ hybridization in the same brains. Results revealed no differences between high- and low-performance rats in NMDA receptor binding in any of the brain areas studied. Similarly, NR2B subunit expression was also not different between groups. However, NR2A expression was significantly higher in the caudate–putamen of low-performance rats. These results suggest that NMDA receptors in the caudate–putamen may also be involved in contextual fear conditioning performance.
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Keywords: Binding; Fear conditioning; In situ hybridization; [^{3}H]MK-801; NMDA; NR2A; NR2B

1. Introduction

Fear conditioning is a Pavlovian paradigm that is widely used to study emotional memory. It can be achieved by pairing an aversive unconditioned stimulus (US, e.g. foot shock) with a neutral discrete stimulus (e.g. a tone) or with the whole context where the animal is at the time of stimulus presentation. These two different forms are known as cued (or tone) conditioning and contextual conditioning, respectively. Distinct neural substrates are believed to contribute to each of these paradigms. Contextual fear conditioning depends on the hippocampal formation and amygdala, whereas tone fear conditioning involves the amygdala but not the hippocampus [16,39].

Both amygdala and hippocampus are rich in NMDA receptors (NMDAr) [7,9,24,25]. NMDAr in the amygdala are required for both tone and contextual fear conditioning [6,17], as well as for long-term potentiation (LTP) in this brain region [3]. LTP is a form of synaptic plasticity which is regarded as a possible cellular mechanism of learning and memory [3], and which can be induced by tone fear conditioning [35]. Hippocampal NMDAr are required only for contextual fear conditioning [2] and for some forms of hippocampal LTP [11].

NMDAr are heteromeric complexes [15], generally composed of at least three subunits, NR1, NR2A and NR2B [21]. Receptor subunit composition can influence learning and memory processes. For instance, NR1 knockout mice show deficits in object recognition, olfactory discrimination and contextual fear memories [34]. Also, absence of the GluRε1 (NR2A) subunit impairs contextual conditioning and increases LTP thresholds [13]. Transgenic mice overexpressing NR2B show improved performance in paradigms such as novel-object recognition test, water maze, cued and contextual fear conditioning and also enhanced hippocampal LTP [41]. LTP changes have also been observed in some lineages of mice that have different performances in tone and/or contextual fear conditioning [26,40]. Altered performance in contextual fear conditioning across different mice lineages have also been reported, suggesting a link

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between different genetic background and conditioned freezing response [1,32].

Another way of exploring this issue is by looking at animals from a single lineage that show high and low freezing responses to the context. Breeding these animals for three generations revealed quantitative trait locus (QTL) differences [33]. However, it is not known if non-selectively bred animals that have different performances in contextual fear conditioning differ in NMDAr subunit expression. Considering that differential subunit expression may interfere with learning, memory and synaptic plasticity, the purpose of this work was to assess brain NR2A and NR2B mRNA expression in rats showing differential sensitivity to contextual fear conditioning. We also examined [^{3}H]MK-801 binding to NMDA receptors in amygdala, hippocampus and other brain areas.

2. Materials and methods

2.1. Subjects

Fifty-eight Wistar male rats, 3–4 months old, bred and raised in the animal facility of the Department of Psychobiology of UNIFESP, were used. Animals were maintained under controlled temperature (23 ± 2 °C) and 12:12 h light–dark cycle (lights on at 7:00 a.m.) conditions. Rat chow and tap water were available ad libitum. All procedures were approved by the UNIFESP Ethics Committee, and followed international guidelines for animal use and care.

2.2. Apparatus

The conditioning apparatus consisted of an acrylic box, measuring 30 cm × 21 cm × 30 cm. The apparatus had black walls with white visual patterns (2 squares measuring 5.5 cm × 5.5 cm, and 3 measuring 4.0 cm × 4.0 cm made of white cardboard. The squares were positioned in a 3 column × 3 row scheme. The larger squares were positioned at the upper left and lower right positions, and the smaller ones at the lower left, central and upper right positions in each wall). The top was covered with transparent acrylic. The floor consisted of a metal grid (0.4 cm diameter rods placed 1.2 cm apart) connected to a shock generator and control module (AVS Projetos Especiais, São Paulo, Brazil).

2.3. Behaviour

The contextual fear conditioning procedure was carried out between 1:00 and 4:00 p.m. every day, with normal light conditions, on four consecutive days. Animals were tested in the same order every day, i.e., each animal was always tested in the same time, to avoid circadian interferences. Animals were individually placed in the conditioning apparatus, where they remained for 3.5 min. After two initial minutes, rats received two footshocks (0.6 mA, 1 s, the US) spaced 30 s apart. They were removed from the apparatus 1 min after the last footshock. Freezing time, defined as time spent in complete immobility, with the absence of vibrissae movements and sniffing [4], was recorded cumulatively by an experienced observer during the 2 min before footshocks were applied.

After the behavioural procedures, animals were classified according to the mean freezing time measured in the two initial minutes of each day. Animals with freezing times below the 25th percentile in each of these 3 days were classified as “Low Performance” (LP) and those above the 75th percentile were defined as “High Performance” (HP). To be classified in one of the groups, animals had to be in the lower or higher 25% of the distribution on days 2–4. Animals not meeting this criterion were not included in subsequent analyses.

Animals were sacrificed on the fifth day by decapitation. Brains were quickly removed, frozen over dry ice and then stored at −80 °C. Serial 20 μm coronal sections were cut in a Leica cryostat at −20 °C. Slides were stored at −80 °C until processing for in situ hybridization and autoradiographic analysis.

2.4. In situ hybridization

Slide-mounted frozen sections were thawed and then prehybridized at room temperature. The sections were fixed in 4% paraformaldehyde for 5 min then rinsed in 1× PBS (2× 5 min). The sections were treated with 0.1 M triethanolamine·HCl for 5 min, then acetylated in 0.1 M triethanolamine·HCl containing 0.25% acetic anhydride for 10 min, followed by two 5 min rinses in 2× SSC. The slides were then dehydrated in graded ethanol, defatted in 100% chloroform for 5 min and hydrated. Hybridization was performed using ^{35}S-UTP labeled riboprobes complementary to the sequences of interest. RNA was extracted from rat brain tissue and cDNA was prepared by reverse transcription using Superscript II enzyme (Invitrogen), primed with Oligo dT. The ^{35}S-labeled cDNA riboprobes were generated by in vitro transcription using the Maxiscript kit (Ambion). During transcription the cDNA was amplified by PCR using compound primers made up of consensus promoter sequences for either SP6 RNA polymerase (atttaggtgacactatagaa) attached at the 5′ end of the left primer and/or for T7 RNA polymerase (taatacgactcactataggg) at the 5′ end to the right primer sequences complementary to the target gene. The labeled riboprobes prepared were complementary to the following sequences: for NR2A, sequences were complementary to Genbank # NM_008170 (bases 1757–1782) and for NR2B complementary to # NM_008171.1 (bases 1971–2016). The ^{35}S-labeled riboprobe was diluted to a concentration of 18,000 cpm/μL in hybridization solution containing: 50% formamide, 35% denhardts, 10% dextran sulfate, 0.1× SSC, salmon sperm DNA (300 μg/mL), yeast tRNA (100 μg/ml), and DTT (40 μM). Slides were incubated in mailers overnight at 60 °C. After hybridization, the sections were rinsed with agitation using decreasing concentrations of SSC containing 25 g/ml sodium thiosulfate. Sections were then rinsed in milliQ water for 10 s, dehydrated in 70% ethanol for 10 s and air-dried. The slides were then exposed to Kodak BioMax film at 4 °C for 2 days. Control sections incubated with sense and scrambled probes showed no signal.

Sections were analyzed using computer-assisted image analysis (MCID, Imaging Research, Inc., St. Catharines, Ont., Canada) using standard curves obtained from calibrated radioactive standards. Anatomical regions were defined according to [31] and sampled without awareness of group membership.

2.5. [^{3}H]MK-801 binding

[^{3}H]MK-801 autoradiographic assays followed published procedures [38] with minor modifications. Briefly, slices were brought to room temperature and then pre-incubated in 50 mM Tris–acetate buffer (pH 7.4) for 30 min at 4 °C. Sections were then incubated for 2 h in buffer containing 5 nM [^{3}H]MK-801 (24.2 Ci/mmol, Perkin-Elmer), at 21 °C. An additional set of slides was incubated in the presence of 20 μM MK-801, for determination of nonspecific binding. Sections were then washed in buffer for 60 min at 4 °C and allowed to dry at room temperature. Slides were exposed to Kodak-Biomax film with calibrated standards for 4 weeks. Films were developed and densitometric analyses performed using an M2 MCID system (Imaging Research, St. Catharines, Ont.). Anatomical regions were defined according to [31] and sampled without awareness of group membership.

2.6. Statistical analyses

Student’s *t*-test for independent samples was used for [^{3}H]MK-801 binding, NR2A and NR2B in situ hybridization. Two-way analysis of variance (ANOVA) followed by post hoc Newman–Keuls was used for behaviour. The behavioural data was expressed as mean freezing time per minute. In contextual fear conditioning, the two-way ANOVA compared two groups (HP and LP) over 4 days.

3. Results

3.1. Behaviour

From the 58 animals trained on contextual fear conditioning, 7 animals were selected as HP and 7 as LP. Analysis of contextual fear conditioning revealed significant group ($F_{1,12}$ =

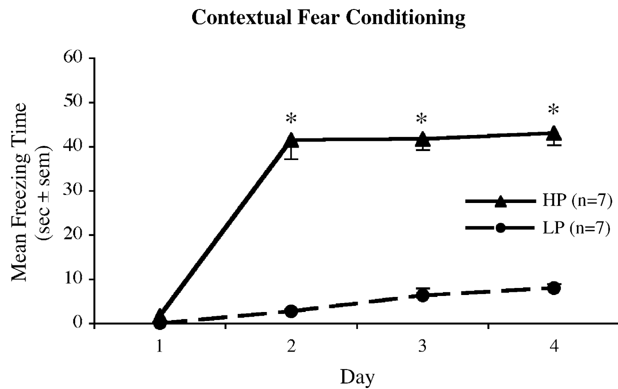

Fig. 1. Mean freezing time per minute of high-performance (HP, $n = 7$) and low-performance (LP, $n = 7$) groups in contextual fear conditioning during four consecutive days. Freezing was measured during 2 min before footshocks every day. Values are expressed as mean ± S.E.M. *indicates difference from LP in each day ($p < 0.01$).

141.42; $p < 0.01$), day ($F_{3,36} = 100.00$; $p < 0.01$) and interaction ($F_{3,36} = 56.46$; $p < 0.01$) effects. Post hoc Newman–Keuls for the interaction showed that the HP group performance was higher on days 2–4 ($p < 0.01$). There were no differences between groups on the first day ($p = 0.46$), before any shock was delivered (Fig. 1).

3.2. Brain analyses

Fig. 2 illustrates NR2A, NR2B and [^{3}H]MK-801 signals. No significant differences between HP and LP groups were seen in any region analyzed for [^{3}H]MK-801 binding (Table 1) or for NR2B in situ hybridization (Table 2). NR2A in situ hybridization revealed significant group differences in the anterior ($p = 0.02$), dorsomedial ($p = 0.02$), dorsolateral ($p = 0.01$) and ventrolateral ($p = 0.02$) parts of the caudate–putamen (Table 3).

4. Discussion

The present results confirm that Wistar rats can be classified in extremes according to freezing behaviour in contextual fear conditioning [33]. It is shown here that when classified on the basis of 3 days of testing, this difference is robust and persists for at least 3 days. Additional experiments in our laboratory revealed that the groups remain different for at least 36 days (data not shown). The current protocol used four conditioning days and allowed the exclusion of animals that showed fluctuations from day to day, such that only those showing consistent behaviour remained.

Brain analyses did not reveal group differences in the expression of NMDA subunits NR2A or NR2B nor in [^{3}H]MK-801 binding in any amygdaloid nuclei, hippocampal formation or in the periaqueductal gray matter. It is well known that lesions [18,20,42] or pharmacological manipulations of these structures with NMDA antagonists [6,10] impair these tasks. Therefore it is surprising that no alterations were found in these regions. Since NMDA and AMPA receptors function are closely related in synaptic plasticity [14,23], one possible mechanism that may account for the behavioural difference observed may be the processes involving AMPA receptors trafficking in synaptic plasticity (for review see [22]). Specifically, long-term potentiation requires AMPA receptor trafficking in the hippocampus

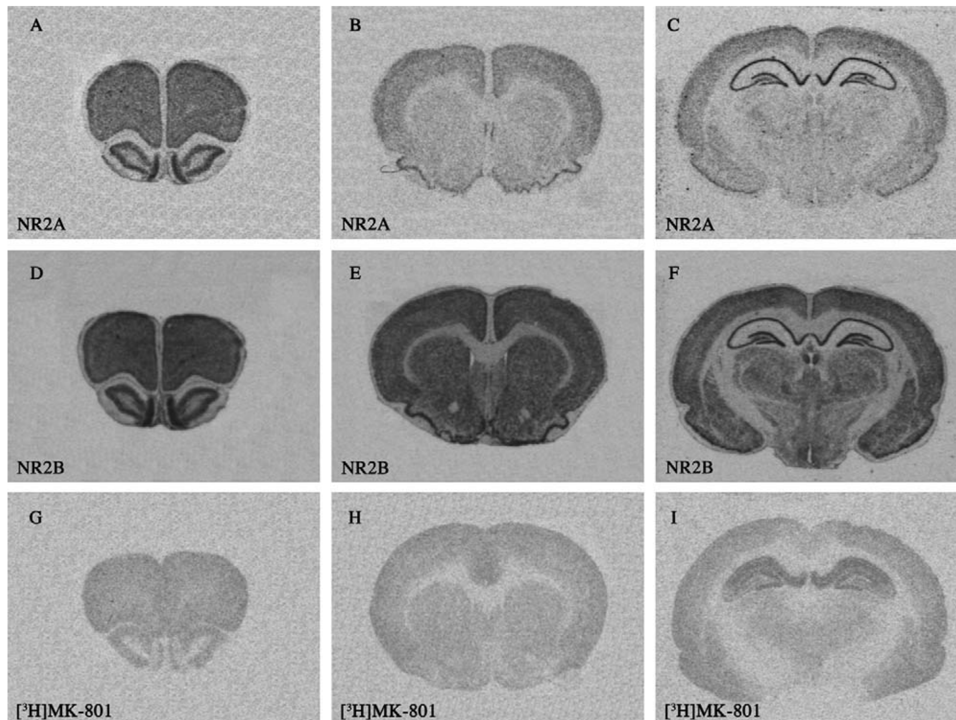

Fig. 2. Photographs illustrating in situ hybridization (A–F) and [^{3}H]MK-801 autoradiography (G–I) patterns in coronal sections. Regions such as frontal cortex (A, D, G), striatum (B, E, H), hippocampus and amygdala (C, F, I) are visible.

Table 1
[^{3}H]MK-801 binding in high- and low-performance rats[a]

Region	High performance ($n=7$)	Low performance ($n=7$)	p
Accumbens n.			
Core	0.35 ± 0.01	0.34 ± 0.02	0.52
Shell	0.32 ± 0.01	0.32 ± 0.01	0.91
Amygdala			
Basolateral n.	0.40 ± 0.02	0.41 ± 0.05	0.81
Basomedial n.	0.37 ± 0.03	0.36 ± 0.05	0.79
Central n.	0.26 ± 0.02	0.27 ± 0.04	0.92
Lateral n.	0.39 ± 0.03	0.40 ± 0.05	0.89
Medial n.	0.25 ± 0.04	0.27 ± 0.05	0.76
Caudate–putamen			
Anterior	0.28 ± 0.01	0.28 ± 0.03	0.79
Dorsolateral	0.27 ± 0.02	0.27 ± 0.04	0.97
Dorsomedial	0.26 ± 0.02	0.25 ± 0.03	0.92
Posterodorsal	0.24 ± 0.02	0.23 ± 0.04	0.79
Posteroventral	0.30 ± 0.02	0.30 ± 0.03	0.93
Ventrolateral	0.28 ± 0.02	0.28 ± 0.03	0.97
Cortex			
Primary auditory	0.42 ± 0.01	0.43 ± 0.03	0.73
Secondary auditory, dorsal associative	0.40 ± 0.01	0.42 ± 0.03	0.51
Secondary auditory, ventral associative	0.43 ± 0.01	0.45 ± 0.03	0.50
Cingulate, areas 1 and 2	0.45 ± 0.02	0.47 ± 0.02	0.60
Entorhinal, external layer	0.45 ± 0.03	0.41 ± 0.06	0.60
Entorhinal, internal layers	0.33 ± 0.03	0.31 ± 0.05	0.84
Frontal, association	0.45 ± 0.03	0.46 ± 0.03	0.87
Orbital	0.46 ± 0.02	0.45 ± 0.02	0.85
Parietal, external layer	0.48 ± 0.03	0.48 ± 0.04	0.93
Parietal, intermediary layers	0.42 ± 0.02	0.42 ± 0.03	0.96
Parietal, internal layers	0.35 ± 0.02	0.35 ± 0.03	0.99
Perirhinal	0.46 ± 0.03	0.49 ± 0.04	0.59
Dentate gyrus			
Crest	0.46 ± 0.03	0.48 ± 0.04	0.82
Internal blade	0.56 ± 0.03	0.56 ± 0.04	0.85
Outer blade	0.57 ± 0.02	0.58 ± 0.04	0.91
Polymorph layer	0.43 ± 0.03	0.45 ± 0.05	0.69
Ventral part	0.48 ± 0.01	0.47 ± 0.02	0.58
Hippocampus			
CA1, dorsal part	0.63 ± 0.02	0.66 ± 0.04	0.57
CA1, ventral part	0.43 ± 0.03	0.42 ± 0.03	0.83
CA3, dorsal part	0.43 ± 0.02	0.44 ± 0.04	0.92
CA3, ventral part	0.48 ± 0.02	0.47 ± 0.02	0.92
Subiculum	0.24 ± 0.03	0.21 ± 0.05	0.70
Hypothalamus			
Medial mammillary n.	0.10 ± 0.01	0.14 ± 0.04	0.27
Thalamus			
Anteroventral n.	0.22 ± 0.02	0.22 ± 0.04	0.95
Central medial n.	0.28 ± 0.02	0.26 ± 0.04	0.76
Geniculate n., dorsolateral	0.27 ± 0.03	0.25 ± 0.05	0.77
Geniculate n., ventrolateral	0.10 ± 0.01	0.13 ± 0.03	0.40
Mediodorsal n.	0.23 ± 0.02	0.23 ± 0.04	0.99
Reticular thalamic n.	0.20 ± 0.03	0.22 ± 0.03	0.64
Ventroposterior n.	0.25 ± 0.02	0.25 ± 0.04	0.93

[a] Values are expressed as mean ± S.E.M. in μCi/g tissue.

Table 2
Expression of NR2B subunit in high- and low-performance rats[a]

Region	High performance ($n=7$)	Low performance ($n=7$)	p
Accumbens n.			
Core	61.82 ± 5.89	61.32 ± 5.77	0.95
Shell	56.99 ± 5.78	61.30 ± 6.30	0.63
Amygdala			
Basolateral n.	55.01 ± 4.76	54.04 ± 4.52	0.89
Basomedial n.	58.18 ± 5.85	57.05 ± 4.50	0.88
Central n.	55.86 ± 5.69	55.75 ± 5.43	0.99
Lateral n.	61.40 ± 6.37	59.82 ± 5.54	0.86
Medial n.	58.82 ± 6.56	60.28 ± 6.99	0.88
Caudate–putamen			
Anterior	51.47 ± 5.42	48.69 ± 3.22	0.66
Dorsolateral	48.00 ± 4.99	45.25 ± 3.23	0.64
Dorsomedial	47.05 ± 4.81	45.06 ± 2.90	0.72
Posterodorsal	44.08 ± 4.45	44.51 ± 3.56	0.94
Posteroventral	46.62 ± 4.38	50.76 ± 4.80	0.54
Ventrolateral	48.59 ± 5.11	47.70 ± 3.41	0.88
Cortex			
Primary auditory	49.62 ± 5.72	50.25 ± 4.76	0.93
Secondary auditory, dorsal associative	45.84 ± 5.01	44.51 ± 4.42	0.85
Secondary auditory, ventral associative	53.41 ± 6.44	54.78 ± 4.86	0.87
Cingulate, areas 1 and 2	73.49 ± 7.65	67.12 ± 4.95	0.49
Entorhinal, external	84.61 ± 8.59	69.01 ± 8.28	0.22
Entorhinal, internal	62.30 ± 6.07	57.46 ± 4.88	0.55
Frontal, association	54.88 ± 6.71	60.11 ± 5.11	0.55
Orbital	55.82 ± 6.80	57.08 ± 3.78	0.87
Parietal, external	99.29 ± 13.71	73.99 ± 6.11	0.14
Parietal, intermediary	70.94 ± 7.27	56.58 ± 4.51	0.14
Parietal, internal	69.46 ± 8.10	55.41 ± 4.89	0.18
Perirhinal	66.38 ± 4.24	61.19 ± 3.60	0.37
Piriform	101.60 ± 11.12	104.30 ± 6.53	0.84
Dentate gyrus			
Crest	144.43 ± 11.26	143.80 ± 5.88	0.96
Internal blade	123.81 ± 12.36	120.50 ± 6.77	0.83
Outer blade	127.04 ± 11.87	124.29 ± 5.02	0.84
Polymorph layer	91.68 ± 10.26	85.55 ± 7.85	0.65
Ventral part	118.68 ± 9.21	114.73 ± 5.18	0.73
Hippocampus			
CA1, dorsal part	107.04 ± 10.59	94.54 ± 7.83	0.38
CA1, ventral part	113.74 ± 8.42	103.57 ± 5.00	0.34
CA3, dorsal part	92.14 ± 9.18	84.83 ± 7.79	0.56
CA3, ventral part	107.68 ± 7.87	101.20 ± 6.42	0.55
Subiculum	54.41 ± 3.91	49.75 ± 3.67	0.40
Hypothalamus			
Medial mammillary n.	47.39 ± 5.49	37.84 ± 5.23	0.24
Periaqueductal gray			
Dorsomedial	36.58 ± 1.09	37.06 ± 4.34	0.92
Dorsolateral	34.46 ± 0.78	33.48 ± 2.84	0.75
Ventrolateral	31.76 ± 1.26	33.18 ± 2.44	0.61
Lateral	32.12 ± 1.19	31.85 ± 2.35	0.92
Substantia nigra			
Pars compacta	40.09 ± 3.00	37.12 ± 2.01	0.43
Pars reticulata	25.26 ± 1.77	23.59 ± 2.41	0.59
Thalamus			
Anteroventral n.	46.16 ± 5.76	48.49 ± 4.18	0.74
Central medial n.	42.95 ± 4.42	41.97 ± 3.32	0.87
Geniculate n., dorsolateral	46.25 ± 3.84	40.92 ± 3.97	0.36
Geniculate n., ventrolateral	42.23 ± 2.74	38.10 ± 3.95	0.40

Table 2 (*Continued*)

Region	High performance ($n=7$)	Low performance ($n=7$)	p
Mediodorsal n.	39.88 ± 3.27	37.62 ± 2.22	0.59
Reticular thalamic n.	45.12 ± 5.68	45.52 ± 4.81	0.96
Ventroposterior n.	41.29 ± 3.15	39.53 ± 3.10	0.70
Ventral tegmental area	33.20 ± 1.92	29.79 ± 2.70	0.32

[a] Values are expressed as mean ± S.E.M. in μCi/g tissue.

Table 3
Expression of NR2A subunit in high- and low-performance rats[a]

Region	High-performance ($n=7$)	Low-performance ($n=7$)	p
Accumbens n.			
Core	11.94 ± 1.15	15.16 ± 1.23	0.08
Shell	13.52 ± 1.04	15.67 ± 1.29	0.22
Amygdala			
Basolateral n.	20.32 ± 0.76	20.81 ± 1.61	0.79
Basomedial n.	19.52 ± 0.50	20.32 ± 1.93	0.69
Central n.	15.98 ± 0.61	17.10 ± 1.53	0.51
Lateral n.	21.01 ± 0.56	21.78 ± 1.88	0.70
Medial n.	18.50 ± 0.92	20.12 ± 2.05	0.49
Caudate–putamen			
Anterior	14.59 ± 0.81	17.54 ± 0.74	0.02*
Dorsolateral	15.01 ± 0.75	18.23 ± 0.80	0.01*
Dorsomedial	14.89 ± 0.77	17.95 ± 0.91	0.02*
Posterodorsal	15.61 ± 0.98	16.96 ± 1.53	0.47
Posteroventral	18.07 ± 0.89	19.56 ± 1.82	0.48
Ventrolateral	16.07 ± 0.69	19.21 ± 0.96	0.02*
Cortex			
Primary auditory	21.73 ± 0.73	22.16 ± 1.29	0.78
Secondary auditory, dorsal associative	21.76 ± 0.80	21.42 ± 1.34	0.83
Secondary auditory, ventral associative	21.77 ± 0.74	22.31 ± 1.28	0.72
Cingulate, areas 1 and 2	22.78 ± 0.97	25.65 ± 1.04	0.07
Entorhinal, external	28.95 ± 1.04	30.61 ± 2.18	0.53
Entorhinal, internal	19.52 ± 0.65	19.71 ± 1.90	0.93
Frontal, association	21.98 ± 1.09	23.91 ± 0.91	0.20
Orbital	21.64 ± 0.93	24.31 ± 1.24	0.11
Parietal, external	26.71 ± 1.52	27.70 ± 2.46	0.75
Parietal, intermediary	21.31 ± 0.87	23.01 ± 1.25	0.30
Parietal, internal	20.67 ± 1.23	21.43 ± 1.41	0.70
Perirhinal	19.68 ± 0.94	22.13 ± 1.42	0.19
Piriform	33.23 ± 1.45	34.75 ± 1.03	0.41
Dentate gyrus			
Crest	55.89 ± 1.79	58.35 ± 4.34	0.61
Internal blade	47.50 ± 2.49	48.24 ± 3.63	0.87
Outer blade	50.76 ± 2.40	50.75 ± 3.33	1.00
Polymorph layer	37.57 ± 1.40	37.23 ± 2.38	0.91
Ventral part	35.54 ± 2.27	37.97 ± 3.05	0.55
Hippocampus			
CA1, dorsal part	56.07 ± 2.10	54.90 ± 2.60	0.73
CA1, ventral part	33.63 ± 1.53	34.90 ± 1.91	0.62
CA3, dorsal part	42.51 ± 1.67	42.39 ± 2.77	0.97
CA3, ventral part	37.63 ± 2.08	40.81 ± 1.89	0.28
Subiculum	21.61 ± 0.85	23.72 ± 1.64	0.30
Hypothalamus			
Medial mammillary n.	17.01 ± 1.12	16.42 ± 1.58	0.77
Periaqueductal gray			
Dorsomedial	14.02 ± 1.22	15.46 ± 1.48	0.48

Table 3 (*Continued*)

Region	High-performance ($n=7$)	Low-performance ($n=7$)	p
Dorsolateral	13.89 ± 0.97	14.70 ± 1.47	0.67
Ventrolateral	12.48 ± 1.26	13.30 ± 1.40	0.67
Lateral	13.11 ± 1.08	14.10 ± 1.53	0.62
Substantia nigra			
Pars compacta	16.76 ± 0.94	16.65 ± 1.14	0.94
Pars reticulata	12.13 ± 0.65	12.18 ± 1.25	0.98
Thalamus			
Anteroventral n.	15.59 ± 1.12	17.47 ± 1.49	0.33
Central medial n.	16.27 ± 0.94	17.41 ± 0.98	0.42
Geniculate n., dorsolateral	15.23 ± 0.89	16.48 ± 1.64	0.53
Geniculate n., ventrolateral	12.45 ± 1.36	14.16 ± 1.70	0.46
Mediodorsal n.	13.80 ± 0.89	14.82 ± 0.78	0.41
Reticular thalamic n.	14.62 ± 0.75	16.31 ± 1.17	0.25
Ventroposterior n.	16.90 ± 0.76	18.50 ± 1.53	0.37
Ventral tegmental area	14.92 ± 0.70	14.73 ± 1.54	0.92

[a] Values are expressed as mean ± S.E.M. in μCi/g tissue.
* Significant differences, independent t tests.

[5]. Recently, tone fear conditioning was shown to drive AMPA receptors to lateral amygdala synapses [37]. This change was observed at approximately 24 h. Since the behavioural difference observed here is long-lasting (at least during 36 days, data not shown) and since AMPA receptors seem to stabilize plasticity [14,23], it remains to be investigated if these changes in AMPA receptors persist for a long-lasting period of time and also if they occur in hippocampus after contextual fear conditioning.

Alterations in NR2B expression in hippocampus were particularly expected, given that NR2B overexpression in this brain area improves memory, including contextual fear conditioning performance [43]. The fact that we found no alterations in hippocampal NR2B mRNAs suggests that the natural variability in fear conditioning performance in rats is not mediated by the same hippocampal mechanism that is involved in the behavioural effect in transgenic mice. As reported [43], other regions besides the hippocampus showed overexpression of NR2B, such as cortex and striatum, raising the possibility that these alterations may be involved in the learning effects observed. In the present study we did not find changes in NR2B expression in striatum but did find alterations in striatal expression of NR2A subunit.

Different subregions of the dorsal striatum, specifically the anterior, dorsomedial, dorsolateral and ventrolateral portions, revealed NR2A alterations. The low-performance group had higher NR2A expression in this region while in [43], high-performance mice have higher NR2B expression in this region. This suggests a possible role for the balance NR2A/NR2B in the striatum and fear conditioning performance. Recent work revealed that selectively blocking NR2B containing receptors in the hippocampus impairs long-term depression (LTD) and spares long-term potentiation (LTP), whereas NR2A blockade impairs only LTP [19]. It would be interesting to verify if this pattern of effects also occurs in the striatum. Several studies demonstrate striatum involvement in learning and memory processes (for review see [44]). It is well known that dorsal striatum

lesions [27,29] or locally injected glutamate antagonists [28,30] impair performance in tasks evaluating stimulus-response learning. Recently, the effects of dorsal striatum manipulations were investigated in Pavlovian fear conditioning. Pre-training electrolytic and neurotoxic dorsomedial lesions impaired tone but not contextual fear conditioning [8], while post-training administration of amphetamine in the dorsolateral striatum improved performance in both tasks [45].

Regional [^{3}H]MK-801 binding alterations have been reported in different learning paradigms. Thus [^{3}H]MK-801 binding was shown to be decreased in hippocampus and frontal cortex after odor discrimination learning [36] and in the amygdala after spatial learning in a radial maze task [36]. Correlations between quantitative NMDA binding and learning ability, an approach similar to that one used in the present study, were analyzed by some authors. When comparing good and poor performers in passive avoidance tasks, good performers revealed higher NMDA receptor density in the hippocampus [41]. Animals inbred for 30 generations for high and low learning capacity in a shuttle box also revealed higher NMDA receptor density in the hippocampus [12]. Our [^{3}H]MK-801 results are not in accordance with these previous findings. Although we found differences in NR2A subunit expression, receptor density was the same in high- and low-performance animals in contextual fear conditioning. Thus NMDA binding may vary as a function of behavioural task used in each experiment.

The approach of comparing animals at both ends of the performance distribution may serve as an additional tool for investigating the neural basis of different aspects of fear conditioning. Our NR2A results point to the striatum as a possible target for future research. Further studies will also be necessary in order to verify whether the observed differences in NR2A striatal expression relate to learning per se or to the somatomotor component of the task.

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