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Research report

Temporal changes in c-Fos activation patterns induced by conditioned fear

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ABSTRACT

Mechanisms underlying shock-induced conditioned fear – a paradigm frequently used to model posttraumatic stress disorder, PTSD – are usually studied shortly after shocks. Some of the brain regions relevant to conditioned fear were activated in all the c-Fos studies published so far, but the overlap between the activated regions was small across studies. We hypothesized that discrepant findings were due to dynamic neural changes that followed shocks, and a more consistent picture would emerge if consequences were studied after a longer interval. Therefore, we exposed rats to a single session of footshocks and studied their behavioral and neural responses one and 28 days later. The neuronal activation marker c-Fos was studied in 24 brain regions relevant for conditioned fear, e.g. in subdivisions of the prefrontal cortex, hippocampus, amygdala, hypothalamic defensive system, brainstem monoaminergic nuclei and periaqueductal gray. The intensity of conditioned fear (as shown by the duration of contextual freezing) was similar at the two time-points, but the associated neuronal changes were qualitatively different. Surprisingly, however, Multiple Regression Analyses suggested that conditioned fear-induced changes in neuronal activation patterns predicted the duration of freezing with high accuracy at both time points. We suggest that exposure to electric shocks is followed by a period of plasticity where the mechanisms that sustain conditioned fear undergo qualitative changes. Neuronal changes observed 28 days but not 1 day after shocks were consistent with those observed in human studies performed in PTSD patients.

1. Introduction

Unavoidable and unpredictable footshocks lead in laboratory rodents to long-term behavioral changes that include hypersensitivity to novelty, startle deficits, behavioral sensitization, anxiety, impaired memory, sleeping problems, hyper-vigilance, and social deficits [16,55,21,45,65,41,42]. For the present study it is important that footshocks result in freezing when subjects are re-exposed to the context or cues previously associated with shocks. These behavioral dysfunctions are reminiscent of human posttraumatic stress disorder (PTSD) symptoms and as such are frequently used to get insights into the mechanisms of this disorder [16,64,50].

One way to study neuronal mechanisms activated in the conditioned fear paradigm is the quantifying of immediate early gene expression, such as c-Fos, usually by means of immunocytochemistry. In spite of some limitations, the use of c-Fos as a marker of neuronal activation remains a powerful tool for identifying activated neurons and extended systems after exposing subjects to particular situations [31]. Laboratory studies employing this technique showed that conditioned fear activates various regions of the medial prefrontal cortex (e.g. the anterior cingulate, and infralimbic cortices), the primary and secondary motor cortices, the occipital and temporal cortices, various regions of the hippocampus (e.g. the CA1 and CA3 area as well as the dentate gyrus), various regions of the amygdala (the basolateral, central, cortical, and medial nuclei) the nucleus accumbens, the paraventricular nucleus of the hypothalamus, certain regions of the periaqueductal gray (e.g. the lateral column), as well as the dorsal raphe and locus coeruleus (Table 1). These findings are in line with observations in humans and suggest that the regions listed above are involved in the expression of conditioned fear, a PTSD-like symptom in laboratory models.

However, the findings become rather confusing when the published papers are compared. After a single series of footshocks and a short incubation time, conditioned fear was associated with increased, decreased as well as unaltered activations, even in the case of the brain regions that are believed to be closest related to conditioned fear (Table 1, A1, B1). More reliable findings were obtained when rats and mice were submitted to shocks several times on consecutive days (Table 1 A2, B2). In these studies, conditioned fear related brain areas consistently showed over-activation when subjects were exposed to the shock-associated context 1–2 days later. However, in the case of these studies one cannot exclude carryover effects from shock days, especially when their number

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Table 1
Conditioned fear-induced changes in c-Fos activation depending on shock exposure and incubation time.

(A) Prefrontal cortex and amygdala

Species	MO	VO	LO	IL	PrL	Cg1	CA1	CA3	DG	CeA	MeA	BLA	Paradigm	Days after shocks*	Reference
A1 - Single exposure to shocks, short incubation time															
Rat										□			Context	1	[52]
Rat						▲	□	□	□	▲	□	□	Ccontext	1	[62]
Rat						□	□	□	□	▲	□	□	Cue	1	[62]
Rat												▲	Context	1	[28]
Rat				□	▲	□				□			Context	1	[40]
Rat							□	□	□	□	□	□	Context	1	[39]
Rat							□	□	□	▲	▲	□	Context	1	[38]
Rat						▲	▲	□	▲	□	□	□	Context	1	[61]
Rat							▼	□					Cue	1	[13]
Rat											▼	▼	Cue	1	[12]
Rat						▲	□	▲	▲	□	▲	□	Context	1	[60]
Rat				▲	▲	▲			▲				Context	1	[27]
Rat						▲	□	□	▲	□	□	□	Context	1	[59]
Mouse						▲				□		▲	Cue	2	[51]
Rat							▲	▲	▲				Context	1	[73]
Mouse				▼	▼							□	Cue	1	[24]
Mouse				□	□	□	▲						Context	1	[17]
Rat				□	□				□	□	□	□	Context	1	[18]
Rat				▲	□				□	□	□	□	Cue	1	[18]
Mouse							▲	□	□				Context	2	[66]
Rat							□		□	▲		▲	Cue	2	[20]
Mouse						□		▲		▲	□	□	Context	1	[43]
A2 - Repeated exposure to shocks, short incubation time															
Mouse			▲	▲		▲	▲		▲	▲		▲	Context	*3* / 7 (4)*	[5]
Rat			▲	▲		▲	▲	▲	▲	▲	▲	▲	Context	*2* / 9 (7)*	[9]
Rat					▲		□		□	▲	▲	▲	Cue	*1* / 21 (20)*	[69]
Mouse								▲	▲	▲	▲	▲	Cue	*2* / 3 (2)*	[33]
Rat						▲					▲	▲	Context	*1* / 4 (3)*	[37]
A3 - Single exposure to shocks, long incubation time															
Mouse				▲	▲	▲	□						Context	36	[17]

(B) Hypothalamic defensive system and brainstem

Species	AH	VMHdm	PMD	PVN	VTA	DR	MnR	LoC	dmPAG	dlPAG	lPAG	vlPAG	Paradigm	Days after shocks*	Reference
B1 - Single exposure to shocks, short incubation time															
Rat									□	□	□	□	Context	1	[52]
Rat				▲									Context	1	[62]
Rat				▲									Cue	1	[62]
Rat				□					□	□	□	□	Context	1	[40]
Rat				□									Context	1	[39]
Rat				▲		▲	▲						Context	1	[38]
Rat				▲									Context	1	[61]
Rat									□	□	□		Context	1	[75]
Rat				▲									Context	1	[60]
Rat												▲	Context	1	[27]
Rat				▲									Context	1	[59]
Mouse				▲				▲					Cue	2	[51]
Rat								□	□	□	▲	□	Context	1	[18]
Rat								□	□	□	□	□	Cue	1	[18]
B2 - Repeated exposure to shocks, short incubation time															
Mouse		▲			▲	▲	▲	▲	▲				Context	*3* / 7 (4)*	[5]
Rat	▲	▲		▲	▲	▲		▲		▲		▲	Context	*2* / 9 (7)*	[9]
Rat				▲		▲	▲						Cue	*1* / 21 (20)*	[69]
Mouse				▲									Cue	*2* / 3 (2)*	[33]
Rat									▲				Context	*1* / 4 (3)*	[37]

*, Days after last shock/days after first shock (number of shock days).
▲, Increased activation; ▼, decreased activation; □, no change; gray cells, not investigated in the particular study.
AH, anterior hypothalamic nucleus; BLA, basolateral amygdala; CA1, field CA1 of hippocampus; CA3, field CA3 of hippocampus; CeA, central amygdala; Cg1, anterior cingulate cortex; DG, dentate gyrus of hippocampus; dlPAG, periaqueductal gray, dorsolateral column; dmPAG, periaqueductal gray, dorsomedial column; DR, dorsal raphe; IL, infralimbic cortex; LO, lateral orbitofrontal cortex; LoC, locus coeruleus; lPAG, periaqueductal gray, lateral column; MeA, medial amygdala; MnR, median raphe; MO, medial orbitofrontal cortex; PMD, premammillary nucleus, dorsal part; PrL, prelimbic cortex; PVN, paraventricular nucleus of the hypothalamus; vlPAG, periaqueductal gray, ventrolateral column; VMHdm, ventromedial hypothalamic nucleus, dorsomedial part; VO, ventral orbitofrontal cortex; VTA, ventral tegmental area.
Notes. We included in this table only those areas, which were studied by us. Certain studies covered other areas as well (e.g. various parts of the sensory and motor cortices, the nucleus accumbens, etc.). Decreases in activation were outlined to increase visibility.