In Vivo Manganese-enhanced MRI of Conditioned Fear Response

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INTRODUCTION

Fear conditioning is widely used to study the neural basis of learning and memory [1]. It allows an organism to quickly assess and react to stimuli and predict danger. However, it was mostly restricted to behavioral or histological studies [2, 3]. Recent studies have been using BOLD-fMRI to detect the neural activity in living animals [4]. However it is technically difficult due to subject motion and is limited by poor spatial resolution. Paramagnetic manganese ion (Mn\textsuperscript{2+}) is known to enter synaptically activated neurons through voltage-gated calcium channels. Based on this, manganese-enhanced MRI (MEMRI) can be used to assess the neural circuitry involved in sensory paradigms [5] and to investigate more complex cognitive and emotional processes such as fear-conditioning longitudinally with higher spatial resolution. In this study, we aim to use in vivo MEMRI to detect the acute neural response after fear conditioning.

MATERIALS AND METHODS

Animal Preparation: Adult C57BL/6N mice (N=9, 90-100 days old, weighing 23-28g) were divided into 2 groups. Animals in Group 1 (N=5) were subjected to fear conditioning (FC), followed immediately by i.p. injection of a 100 mM solution of MnCl\textsubscript{2} in isotonic saline at a dose of 45 mg/kg. Group 2 (N=4) served as controls with no fear conditioned stimulus but the same Mn administration dosage. MRI scans were performed on all the mice before and 24 hours after Mn injection. Conditioning protocol [6]: On the training day, mice were placed individually into a conditioning chamber (25×25×25cm\textsuperscript{3}) for 5 min, followed by 3 paired presentations of a clicker (30 sec, 4Hz, 80 dB) and footshock (2 sec, 0.5 mA). The inter-trial interval was 2 min and an additional 2-min rest after the final clicker/shock pairing in the chamber, yielding a total training time of 15min30s. The chambers were cleaned with 70% alcohol between each training session. MRI Protocols: All MRI experiments were performed on a Bruker 7 T scanner with a 40 cm bore magnet. All MRI experiments were performed on a Bruker 7 T scanner with a MTX = 256×256, slice thickness = 0.5 mm, number of slices = 10, TR/TE = 420/7.5 ms, RARE factor = 4 and NEX = 64; T2WIs were acquired using the same voxel dimensions and slice geometry with TR/TE = 4200/36ms, RARE factor = 8 and NEX = 2.

RESULTS AND DISCUSSION

With the present dosage of Mn administration, no animals were found to have significant Mn-induced behavioral disturbances due to its toxicity. Significantly decreased locomotor movement and increased freezing duration were found during FA training confirmed the mice acquired associative learning with aversive stimulus quickly (data not shown). The Mn-enhanced T1W images in Fig. 1 (left & middle) showed that the FC animals exhibited a higher Mn uptake in several brain regions as indicated by the pointing arrows, including amygdala, hippocampus, paraventricular nucleus of hypothalamus (PVH), and cingulate cortex comparing to the normal controls. The color-coded ratio map between the two groups (Fig.1, right) further delineated the differences of Mn-enhancement in these regions. All these structures were closely related to the process of conditioned fear [7]. For example, the amygdala is playing a key role in the formation and expression of fear while the fear memory is stored in the hippocampus. Fig. 2 showed a significantly higher Mn-uptake in FC animals in amygdala, hippocampus and PVH, possibly due to the fear-induced cellular hyperactivity in these regions. Exceeding the pain threshold [6] with the stimulation current during footshock may also result in the greater Mn-enhancement in PVH [8] in Group 1. Cingulate cortex, which plays a role in acquisition and extinction of conditioned-fear, also showed an increased Mn enhancement after Mn-injection between the FC animals and controls, indicating the regional enhancement was specific to fear-conditioning. In conclusion, this study demonstrated that the neural response after the induction of conditioned-fear can be detected in most brain regions that are highly related to the process of fear by in vivo MEMRI. The results provide insights into neurocircuits involved in fear-conditioning and consolidate the capability of MEMRI as an in vivo probe for mapping neural activity.

REFERENCES