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Case report

Identifying non-toxic doses of manganese for manganese-enhanced magnetic resonance imaging to map brain areas activated by operant behavior in trained rats

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ABSTRACT

Manganese-enhanced magnetic resonance imaging (MEMRI) offers unique advantages such as studying brain activation in freely moving rats, but its usefulness has not been previously evaluated during operant behavior training. Manganese in a form of $MnCl_2$, at a dose of 20 mg/kg, was intraperitoneally infused. The administration was repeated and separated by 24 h to reach the dose of 40 mg/kg or 60 mg/kg, respectively. Hepatotoxicity of the $MnCl_2$ was evaluated by determining serum aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin and protein levels. Neurological examination was also carried out. The animals were tested in visual cue discriminated operant task. Imaging was performed using a 3T clinical MR scanner. T1 values were determined before and after $MnCl_2$ administrations. Manganese-enhanced images of each animal were subtracted from their baseline images to calculate decrease in the T1 value (ΔT1) voxel by voxel. The subtracted T1 maps of trained animals performing visual cue discriminated operant task, and those of naive rats were compared. The dose of 60 mg/kg $MnCl_2$ showed hepatotoxic effect, but even these animals did not exhibit neurological symptoms. The dose of 20 and 40 mg/kg $MnCl_2$ increased the number of omissions and did not affect the accuracy of performing the visual cue discriminated operant task. Using the accumulated dose of 40 mg/kg, voxels with a significant enhanced ΔT1 value were detected in the following brain areas of the visual cue discriminated operant behavior performed animals compared to those in the controls: the visual, somatosensory, motor and premotor cortices, the insula, cingulate, ectorhinal, entorhinal, perirhinal and piriform cortices, hippocampus, amygdala with amygdalohippocampal areas, dorsal striatum, nucleus accumbens core, substantia nigra, and retrorubral field. In conclusion, the MEMRI proved to be a reliable method to accomplish brain activity mapping in correlation with the operant behavior of freely moving rodents.

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ACb, nucleus accumbens; AHi, amygdalohippocampal area; AI, agranular insular cortex; AMY, amygdala; Au, auditory cortex; Cg, cingulate cortex; CPu, caudate – putamen; DI, dysgranular insular cortex; Ect, ectorhinal cortex; Ent, entorhinal cortex; GI, granular insular cortex; GP, globus pallidus; Hip, hippocampus; Hy, hypothalamus; Icj, islands of Calleja; M1, primary motor cortex; M2, secondary motor cortex; MeA, medial amygdaloid nuclei; Pir, piriform cortex; Pit, pituarity; PRh, perirhinal cortex; PtA, parietal association cortex; RRF, retrorubral field; RS, retrosplenial cortex; RSA, retrosplenial agranular cortex; RSG, retrosplenial granular cortex; S, subiculum; SN, substantia nigra; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; TeA, temporal association cortex; Tu, olfactory tubercle; VPm, ventral posteromedial thalamic nucleus; VPl, ventral posterolateral thalamic nucleus; V1, primary visual cortex, binocular area; V2, secondary visual cortex, lateral area.

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1. Introduction

Two basic classes of conditioned responses, the Pavlovian and operant behavior are being studied since the 20th century to understand neuronal control of motivated, reward-related behavior. Whereas examination of Pavlovian conditioned behavior helps us to understand the effects of the learned contingency between environmental, primary and secondary reinforcing stimuli, a performance of operant conditioned tasks reflects processes that control actions [1–7]. The brain regions involved in the control of the operant behavior are examined in increasing number of studies using rodent's models. Cortico-striato-limbic network has been described, which mediates the control over goal-directed behavior, habits, and reward-related processes like the effects of goal value or Pavlovian conditioned motivation on the operant behavior [1–4,8–10]. The role of the parallelly organized cortico-dorsal striato-pallido-thalamic loops with limbic structures and mesencephalic dopaminerg regions in the regulation of the operant behavior was investigated by lesion techniques [1,3,4], pharmacological manipulations [8,11], and electrophysiological studies [9,10] in rodents. Imaging of the brain activity during specific operant tasks in behaving rats could have the benefit to detect this network and to give possibility for further investigation of its role in the action control.

The manganese-enhanced magnetic resonance imaging (MEMRI) is an in vivo technique to map brain activities in small animals usually in mice and rats [12–30]. The major benefit of this method is that it provides opportunity for the whole-brain approach like it is in the case of the fMRI or PET. The presentation of the stimuli or behavioral events is not limited by anaesthesia such as in the case of the blood-oxygen-level-dependent fMRI. It is also worth noting that, the access to the MEMRI as an MRI technique is more commonly available than to the high resolution PET. The MEMRI is based on that manganese ions (Mn^{2+}) pass through the blood brain barrier and can enter into excited cells among others via voltage-gated calcium channel [31,32]. The excited neurons accumulate them in an activity-dependent manner while shortening the T1 and T2 relaxation time [23,24,33–36]. Based on the slow 17–22 days long elimination of Mn^{2+} from the cell allows imaging to be performed following the behavioral responses of animals [33,34,36]. The Mn^{2+} is a unique MRI contrast agent enabling a cytoarchitectonic visualization [21,33,37,38]. Local injection of the ions into the brain gives an opportunity to detect anterograde functional connections [6,18,28,39–41]. The technique can image brain areas activated by sensory [23,42,43] or pharmacological stimulation [23,43,44] in anesthetized animals. Moreover, the MEMRI can display activated brain areas in relation to voluntary activity [12] or responses to unconditioned [14,17–19,27] or conditioned stimuli [16] in awake freely moving animals using a local or systemic injection of Mn^{2+}. Because, MEMRI offers unique advantages studying brain activation in freely moving rats, we hypothesized that this imaging method is able to detect the cortico-striatal-limbic network activated during operant behavior. Our goal was to extend the application of the MEMRI technique for operant behavior.

The main disadvantage of this method is the toxicity of Mn^{2+} [31,45]. The reported symptoms by using manganese during MEMRI in rats are lethargy [33,46,47], hypophagia, loss in body weight [12,22,46] decreased locomotor activity [12]. In the MEMRI experiments, the appropriate systemic doses for rats are in the range of 16–180 mg/kg applying via different routes (i.e. intravenous, intraperitoneal and subcutaneous) by single or repeated injection or slow infusion using an osmotic pump. These studies indicate that the dose <80 mg/kg $MnCl_2$ in the form of single injection is without neurotoxic effect [12,22,48]. However, >95% of this metal gets eliminated through biliary excretion, and the overexposure of Mn^{2+} may lead to hepatotoxicity [31,49]. Moreover, low dose of Mn^{2+} may cause intrahepatic cholestasis in case of increased bilirubin concentration [31,50,51]. Cholestasis, in turn, should reduce the elimination of Mn^{2+}, enhancing its toxic effect limiting its applicable dose. Because no data has been reported so far about the hepatotoxic effect of Mn^{2+} at the doses which are applied during MEMRI and the resulted gastrointestinal dysfunction may have an impact on the motivation during the operant behavior [1,2], serum concentrations of total protein, albumin, total bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level were measured as characteristic parameters on the liver functions.

In human patients, Mn^{2+} overexposure causes manganism, a Parkinson's like disease with symptoms of bradykinesia, rigidity, postural instability, tremor and cognitive deficits [31,45,52,53]. In rodents, the Mn^{2+} accumulation led to subsequent basal ganglia dysfunction [53–55] and among others causes reduced locomotor activity and impaired motor coordination in rats after long-term manganese exposure [55]. It is known that microlesion of the globus pallidus is enough to result in ataxia, impaired motor coordination with pathological reflexes and complex motivational and motor deficiency with difficulties in the orientation toward sensory stimuli [56]. Based on these data and that the basal ganglia have a role in the execution of the operant responses [1], neurological examination is required to observe the sensory and motor functions after Mn^{2+} application. The dose of 40 mg/kg $MnCl_2$ does not have an effect on the one-trial place learning in the delayed-matching-water-maze paradigm [22]. 80 mg/kg systemic dose of $MnCl_2$ did not influence the choice accuracy in the T-maze delayed alternation task, but it increased latency time, decreased body weight and voluntary motor activity [12,48]. We examined the effects of the Mn^{2+} on the operant behavior using the visual cue discrimination task. The visual cue discriminated operant task is a two-lever instrumental situation, which requires detection of the signal, discrimination between the signalized or non-signalized lever, execution of motor response and fast and repeated processing based on high event rate including sustained attention [57–59]. Based on those attributes, the examination the effects of a Mn^{2+} on the visual cue discriminated operant test, should give further data about the influence of the metal ion on cognition.

In the present experiments, first, we have determined the highest applicable dose of the Mn^{2+} with the lowest toxicity for hepatic function, sensory and motor system and operant behavior. We applied the dose of 20 mg/kg $MnCl_2$ intraperitoneally. Because, the fractioned form of treatment can avoid or reduce the toxic effects [22,24,46] the infusion was repeated. The cumulative dose of 40 or 60 mg/kg $MnCl_2$ was reached to have a detectable dose of Mn^{2+} for the imaging. The change in T1 relaxation time was measured to test the different doses of $MnCl_2$ using a 3T clinical scanner. Next, the MEMRI protocol was tested to detect the brain activity (i.e. decrease in T1 relaxation time) induced by the visual cue discriminated operant behavior in comparison to the brain activity in naive control rats.

2. Materials and methods

2.1. Animals and care

73 adult (12 weeks old, weighing 250–280 g at the beginning of the experiment) male Wistar rats participated in the experiments. In 38 rats, effect of $MnCl_2$ injection was examined on liver functions and sensory-motor capabilities ($n = 24$), or on operant behavior ($n = 14$). Imaging was not performed at all in these animals. Another group of 35 animals was used for imaging in the MEMRI experiments, and out of them 16 animals participated in the evaluation of the MEMRI protocol and 19 animals were in the experiment to map the brain areas activated by operant behavior. During the experiments, rats were individually caged in a temperature controlled (24 ± 2 °C) vivarium with 12 h light-dark illumination cycle (light on at 6 a.m.). Standard laboratory food pellets (11.90 MJ/kg, CRLT/N pellets for rodents, HU 13 1 00039, Charles River Kft., Hungary) and tap water were available ad libitum. Daily water consumption and body weight were measured at 8.00 a.m. to the nearest gram and milliliter, respectively. Subjects for operant situation and its controls were maintained on 22 h water