

# c-Fos induction in visceral and vestibular nuclei of the rat brain stem by a 9.4 T magnetic field

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Recently, it has been shown that rats placed in a 9.4 T static magnetic field for 30 min after drinking a glucose-saccharin solution develop a conditioned taste aversion (CTA) to glucose-saccharin. We sought to identify brain stem regions that are activated by the 9.4 T magnetic field exposure using c-Fos immunohistochemistry. Rats were restrained in a 9.4 T magnet for 30 min; sham-exposed rats were restrained but not

exposed to the magnetic field. The magnetic field induced significantly more c-Fos-positive cells than sham treatment in the solitary tract, parabrachial, medial vestibular, prepositus, and supragenualis nuclei. These results suggest that magnetic field exposure causes neural activation in visceral and vestibular nuclei that may promote CTA learning. *NeuroReport* 11:2681-2685 © 2000 Lippincott Williams & Wilkins.

**Key words:** Gene expression; Immediate-early gene; Magnetic resonance imaging; Rotation; Taste aversion

## INTRODUCTION

Advances in MRI are driving the development of MRI machines with more power and higher resolution. While MRI machines with static magnetic fields (MFs) of 1–2 T and resolutions of 2 mm<sup>3</sup> are standard in clinical use, higher resolution requires stronger MFs: 4–9 T MRI machines are becoming available to achieve submillimeter resolutions. Although there is evidence that lower vertebrates can detect small gradients in weak, earth-strength magnetic fields (~50 µT) [1], and the biological effects of oscillatory magnetic fields are well-established [2], little is known about the sensory or physiological effects of high-strength, static magnetic fields on mammals. There have been reports, however, of sensory and visceral disturbances in humans exposed to high MFs. Some effects are transient and purely sensory, such as the phenomenon of magnetophosphenes: the perception of flashing light specks induced by direct stimulation of retinal cells by magnetic fields [3]. More significant are reports of vertigo and nausea reported by workers using large magnets, e.g. during a safety study of an early 4 T MRI machine [4].

Recently, it was found that exposure to 9.4 T magnet can induce a conditioned taste aversion (CTA) in rats [5]. CTA is a form of associative learning by which an animal avoids a novel taste or food that has been previously paired with a treatment that induces visceral or vestibular distress. In the previous study, rats were given a palatable glucose-saccharin solution to drink immediately prior to 30 min restraint either within the bore of a 9.4 T magnet or during sham-exposure with identical restraint outside the magnet.

Upon removal from the magnet, most rats were observed to turn in tight circles during locomotion in the home cage, as if after whole-body rotation (Nolte and Pittman, personal communication). Rats received three pairings on three consecutive days of glucose-saccharin and magnet or sham exposure. Subsequently, sham-exposed rats consumed more glucose-saccharin solution than water during two-bottle, 24 h preference tests. The magnet-exposed rats, however, acquired a CTA and showed a profound aversion to the glucose-saccharin solution that persisted for at least 7 post-exposure extinction tests.

CTA has been widely used as a marker of aversive effects of drugs and other treatments. CTA learning is remarkably sensitive and often reveals a treatment effect even when no other behavioral effect is detectable. Aversive stimuli that activate interoceptors or induce nausea in humans are favored to induce CTA [6]; for example, rotation and motion sickness can induce CTAs [7–9]. Thus, the observation that a 9.4 T magnetic field induces a CTA in rats suggests that high-strength magnetic fields can cause visceral or vestibular distress that may be correlated with human self-reports of nausea and vertigo caused by magnetic fields.

In this experiment we examined the pattern of c-Fos expression in the rat brain stem induced by 30 min exposure to a static, 9.4 T magnetic field to determine if visceral or vestibular nuclei of the brain stem are activated by magnetic field exposure. We employed the same 9.4 T magnet and exposure time that was sufficient to induce a CTA in the earlier study. The c-Fos immediate-early gene

can be induced in central neurons by sensory stimulation, and c-Fos expression has been widely used as a correlate of neuronal activity to map central pathways involved in sensory processing. The brain stem circuitry involved in the processing of vestibular and visceral sensation is well known in outline, including relays in vestibular nuclei, the dorsal vagal complex, and the parabrachial nucleus. The patterns of c-Fos expression induced by rotation or other vestibular manipulations have been described [10,11]; they overlap with the patterns of c-Fos expression induced in visceral nuclei by physiological or toxic visceral stimuli [12–14]. Thus, by examining the pattern of c-Fos expression within the brain stem we can begin to elucidate the sensory pathways through which the 9.4 T field produces a CTA in rats.

## MATERIALS AND METHODS

**Animals:** Twelve male Sprague–Dawley rats were housed individually in transparent plastic cages in a temperature-controlled portable environmental chamber at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL. Rats were maintained on a 12:12 h light schedule and had *ad lib* access to standard chow (Purina 5001) and deionized water.

**Magnetic field exposure** A superconducting Oxford Instruments 400/89 magnet was used for magnetic field exposure at the NHMFL. This magnet has a fixed-field strength of 9.4 T and a vertical bore 6.7 cm in diameter. Measured along the vertical axis of the bore, the magnetic field degrades from 9.4 T at the center to 9.3 T over  $\pm 11$  cm; measured horizontally from the center of the bore, the field degrades to 10 Gauss (1 T = 10 000 Gauss) within 2.4 m. The magnetic field is oriented vertically with the north pole at the bottom of the magnet.

During exposure or sham treatment, all rats were restrained in a Plexiglas tube of 5 cm diameter. The top of the tube was fitted with a cone-shaped plug to accommodate the rat's head; a 1 cm hole was drilled at the cone's apex to allow for breathing. Another open-centered plug was inserted into the bottom of the tube to restrict movement while accommodating the rat's tail. For magnet exposure, the rat in the Plexiglas tube was placed on a pedestal of PVC tubing, inserted into the magnet, and elevated to its core. For sham exposure, the chamber was covered with an opaque PVC tube with similar dimensions as the bore of the magnet. The sham exposure was conducted in the same room as the magnet, but beyond a line marking a field strength of  $< 10$  Gauss.

On test day, rats were carried in their home cages from the animal room to the magnet laboratory ( $\sim 100$  m distance) one at a time. Rats in the magnet group ( $n = 6$ ) were placed in the restraint tube, inserted upward into the bore of the magnet, and exposed to the 9.4 T magnetic field for 30 min. Sham-exposed rats ( $n = 6$ ) were placed in the restraint tube and covered with the opaque sham tube outside the 10-Gauss line for 30 min. After 30 min restraint, rats were immediately released into their home cages in the same room as the magnet; the rats were observed for the occurrence of counter-clockwise turning during locomotion. Rats remained undisturbed in their home cage in the magnet room until perfusion. In order to allow time for tissue

collection, rats were exposed individually at 30 min intervals, alternating between magnet and sham exposures. Exposures were conducted during early to mid-light period.

**Tissue processing:** One hour following magnet exposure or sham restraint, rats were overdosed with sodium pentobarbital. Once completely unresponsive, rats were transcardially perfused, first with 100 ml heparinized isotonic saline containing 0.5%  $\text{NaNO}_2$ , then with 400 ml 4% paraformaldehyde in 0.1 M sodium phosphate buffer. Brains were removed, blocked, post-fixed for 2 h and transferred into 30% sucrose for cryoprotection. Coronal sections were cut at 40  $\mu\text{m}$  on a freezing, sliding microtome. Eighty sections were cut through the medulla from the caudal subpostremal end of the nucleus of the solitary tract (NTS; bregma  $-14.1$  mm) to the rostral extent of the medial vestibular nucleus (MeV; bregma  $-11.6$  mm). In addition, 50 sections were cut through the pons from the caudal supragenualis nucleus (SG; bregma  $-10.5$  mm), through the locus coeruleus (LC) to the rostral tip of the lateral parabrachial nucleus (latPBN; bregma  $-9.0$  mm; coordinates from Paxinos and Watson [15]).

Alternate tissue sections were processed for c-Fos-like immunohistochemistry (c-FLI). Free-floating sections were washed twice in 0.1 M sodium phosphate-buffered saline (PBS), then permeabilized in 0.2% Triton, 1% bovine serum albumin (BSA) in PBS for 30 min. After two washes in PBS-BSA, sections were incubated overnight with a rabbit anti-c-Fos polyclonal antiserum raised against human c-Fos residues 4–17 (Oncogene Sciences Ab-5, 1:20 000 dilution). After incubation for 1 h with a biotinylated anti-rabbit goat antibody, bound secondary antibody was amplified with a Vector Elite ABC kit. Antibody complexes were visualized by a 5 min reaction with 0.05% diaminobenzidine.

**Data analysis:** Images of brain regions ( $720 \times 540 \mu\text{m}$ , 1.1 pixels/ $\mu\text{m}$ ) were digitized with an MTI CCD72S grayscale camera mounted on an Olympus AX70 microscope. Cells expressing positive c-Fos immunohistochemistry were counted automatically by a Macintosh image analysis program (MindsEye). Cells were counted in several visceral nuclei, including NTS (mean of 19 sections per rat), area postrema (AP; five sections), LC (seven sections), and latPBN (eight sections). Stained cells were also quantified in MeV (11 sections per rat), nucleus prepositus (Prp; 11 sections), and nucleus supragenualis (SG; eight sections), all of which express c-Fos after vestibular stimulation. Bilateral cell counts for each section were averaged for each rat; the mean counts were then averaged across rats in each experimental group. Data were analyzed by ANOVA, with significance set at  $p = 0.05$ .

## RESULTS

**Turning behavior:** When removed from the core of the 9.4 T magnet, four of the six rats exposed to the magnet for 30 min turned in tight, counterclockwise, nose to tail circles 4–5 times immediately upon release from the restraint tube, as if they had experienced whole body rotation or other vestibular stimulation. None of the control rats demonstrated this behavior. This confirmed the earlier anecdotal report of turning after 9.4 T exposure (Nolte and Pittman, personal communication).

**Visceral relays:** Magnetic field exposure induced significantly more c-FLI than did sham exposure in the NTS and latPBN (Fig. 1, Fig. 3). In the NTS, c-FLI was abundant in the medial NTS, in the intermediate, subpostremal, and caudal NTS; little or no c-FLI was observed in the lateral or rostral (gustatory) NTS. Some c-FLI was seen within the AP and along the NTS-AP border in magnet-exposed rats, but there was no significant difference overall between the AP in magnet-exposed and control rats. In the latPBN, c-FLI was concentrated in the external lateral, central lateral, and ventrolateral regions. Many c-FLI-positive cells were seen in the LC of both magnet-exposed and sham-exposed groups, consistent with the effects of restraint stress experienced by both groups.

**Vestibular nuclei:** In addition to inducing c-FLI in visceral afferent relays, magnet exposure increased c-Fos expression in nuclei associated with the vestibular system (Fig. 2, Fig. 3). In particular, c-FLI-positive cells were densely induced by magnet exposure in the Prp and SG. Within the MeV, the pattern of c-FLI-positive cells was diffuse, but significantly increased in magnet-exposed rats compared to control rats.

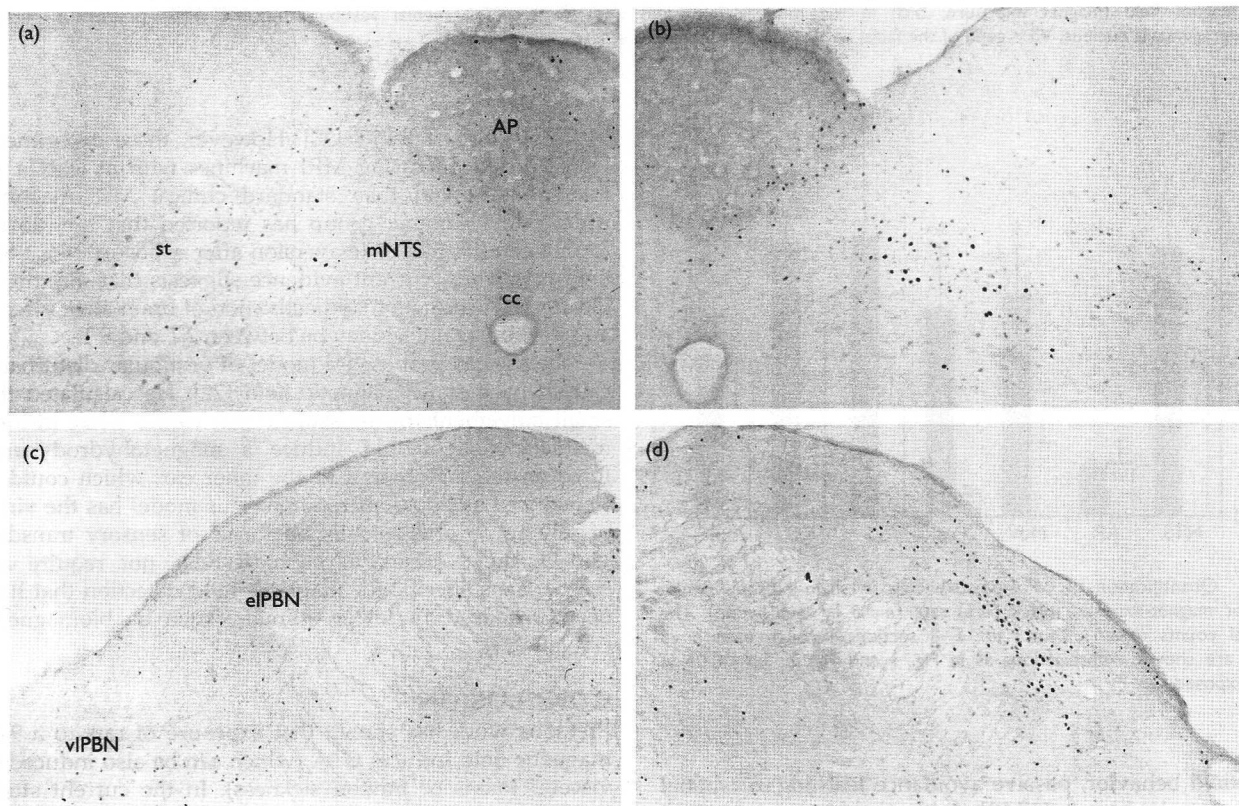
## DISCUSSION

We observed increased c-Fos induction in visceral and vestibular relays of the rat brain stem after 30 min exposure to a 9.4 T static magnetic field. The induction of c-Fos in

the brain stem was not caused by restraint, because sham-exposed control rats were also restrained for 30 min but expressed little or no c-FLI in most brain stem nuclei. Furthermore, most of the rats exposed to the magnetic field rotated counterclockwise when removed from the magnet, suggesting a transient vestibular disturbance. Because the induction of c-Fos protein is a marker of neuronal activity, these results demonstrate activation of brain stem sensory relays and thus provide functional evidence of magnetic field detection by rats complementary to earlier behavioral evidence. Finally, because vestibular and visceral stimuli can mediate the acquisition of condition taste aversions, these results are consistent with the earlier observation that multiple pairings of a taste with 30 min exposure to a 9.4 T static magnetic field (but not 30 min restraint alone) can produce a CTA in rats.

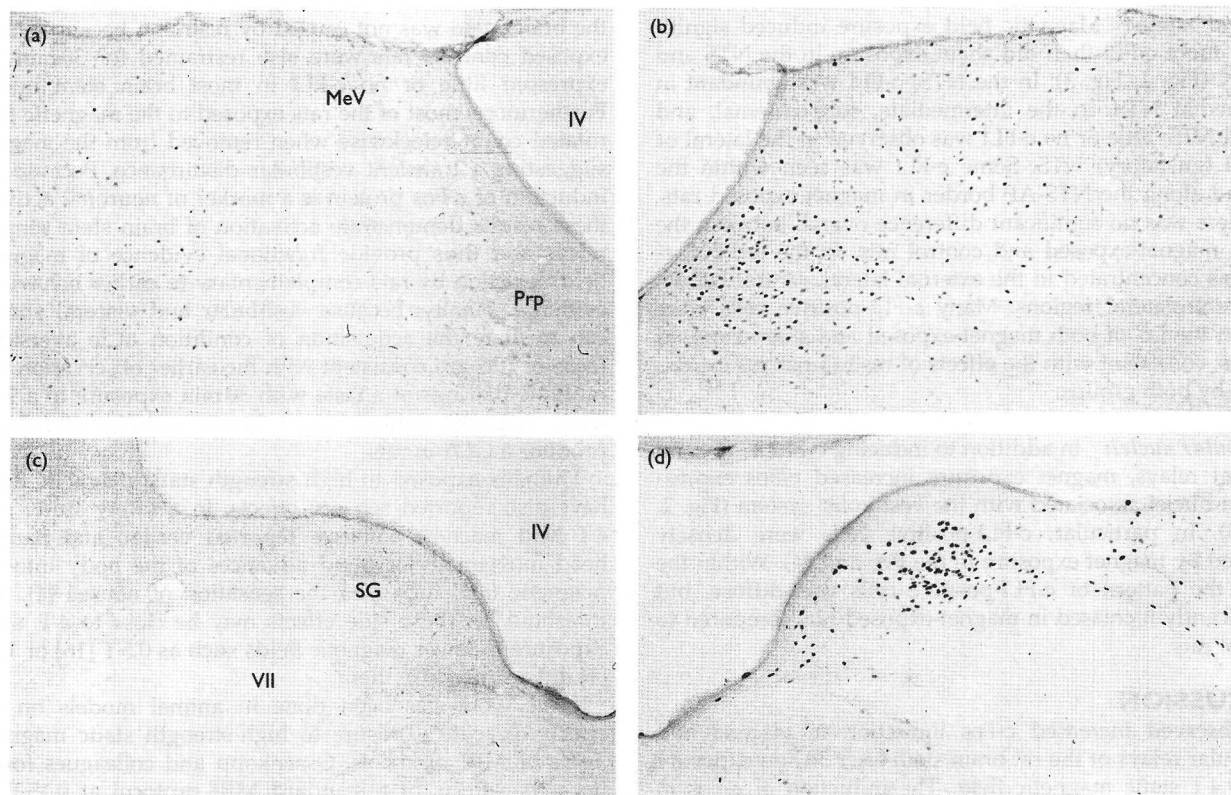
Humans exposed to high strength static magnetic fields have also reported sensory effects. In a safety study of a 4 T MRI machine, subjects reported vertigo and nausea; head movements or rapid advances of the body into the magnetic field increased the sensation of nausea [4]. The threshold for these side effects may be close to 4 T, since exposure to lower magnetic fields such as 0.5 T [16] or 1.5 T [4] did not produce them.

Little work has been done in animal models on the effects of acute exposure to high-strength static magnetic fields or MRI protocols. Ossenkopp and colleagues found no acute effects of a standard MRI protocol at 0.15 T on

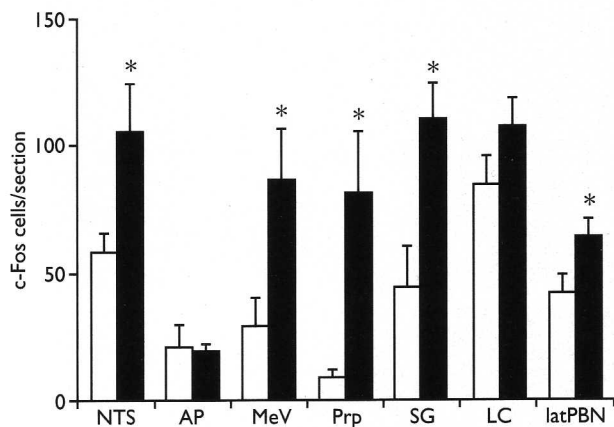


**Fig. 1.** Examples of c-Fos induction in visceral nuclei of the rat brain stem after 30 min restraint (sham exposure, a,c) or 30 min restraint in a 9.4 T magnet (magnet exposure, b,d) in the dorsal vagal complex (a,b) and parabrachial nucleus (c,d). AP = area postrema, NTS = nucleus of the solitary tract, cc = central canal, IV = fourth ventricle, eIPBN = external lateral parabrachial nucleus, vIPBN = ventrolateral PBN, bc = brachium conjunctivum.





**Fig. 2.** Examples of c-Fos induction in vestibular nuclei of the rat brain stem after 30 min restraint (sham exposure, a,c) or 30 min restraint in a 9.4 T static magnet field (magnet exposure, b,d) in the medulla (a,b) and pons (c,d). MeV=medial vestibular nucleus, Prp=prepositus nucleus, SG=supragenualis nucleus, VII=genu of the facial nerve, IV=fourth ventricle.



**Fig. 3.** Quantification of c-Fos-positive cells in sham-exposed (white bars) or magnet-exposed (black bars) rats ( $n=6$  in each group). The bilateral counts (mean  $\pm$  s.e.m.) for 5–8 sections through each brain region are shown. Abbreviations as in Fig. 1 and Fig. 2. \*  $p < 0.05$  vs sham-exposed rats.

open-field behavior, passive avoidance learning, or spatial memory tasks in rats [17,18]; further, no long-term effect on organ pathology and blood chemistry was found 13–22 months after exposure [19]. The same group has reported an attenuation of morphine-induced analgesia in mice after

MRI exposure at 0.15 T [20]. However, these experiments were carried out using MRI machines with at least a 10-fold weaker field than standard clinical MRI machines used today. Another group has reported that rats do not form a conditioned taste aversion after exposure to a 1.89 T field [21]. Thus, current evidence suggests that the threshold for CTA induction and activation of brain stem visceral and vestibular nuclei may be between 2 T and 9 T.

Schenck has proposed a model of vestibular disturbance caused by a static magnetic field [22]. He calculated that small movements of the semicircular canals while inside a magnetic field would induce a magnetohydrodynamic force on the endolymph of the inner ear, which could be transduced as apparent rotation. This model has the virtue of relying on well-established forms of sensory transduction in the vestibular system; it does not require any mechanisms specific for magnetic field detection that have been proposed in lower animals, such as biomagnetite crystals [23].

## CONCLUSION

Previous work has shown that exposure of rats to a 9.4 T magnetic field induces CTA (which can be also induced by visceral toxins or motion sickness). In the current study, restraint with exposure to a 9.4 T magnetic field induced significantly more c-Fos expression than restraint alone in vestibular and visceral nuclei of the brain stem (similar to the c-Fos patterns induced by rotation). We conclude that a

9.4 T magnetic field may activate vestibular or visceral sensory pathways in the rat, and that this stimulation is sufficient to mediate a CTA when the 9.4 T magnetic field is paired with a novel taste. Schenck's model of vestibular disturbance at the level of the semicircular canals provides a working hypothesis for the mechanism by which rats detect a magnetic field. Although we hypothesize that the labyrinth of the inner ear is the site of magnetic field detection, other sensory pathways may contribute to visceral stimulation mediating CTA acquisition. The other two major pathways contributing to CTA learning are subdiaphragmatic vagal afferents from the gut [24] and the chemoreceptive area postrema [25]. Lesion studies will be required to determine a functional role for the vestibular system or other pathways for static magnetic field detection in rats.

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