Supporting Online Material

Materials and Methods

Subjects

Participants were seventeen healthy subjects (10 women) in both experiment 1 (mean age 25 ± 3 yr) and experiment 2 (mean age 27 ± 6 yr), eleven of them participating to both experiments. All were right-handed, had normal vision, no past othological, neurological or psychiatric history and no structural brain abnormalities. Written informed consent was obtained according to the procedures approved by the Ethics Committee of the Scientific Institute Foundation Santa Lucia, Rome.

Tasks and protocols

EXPERIMENT 1: VISUAL MOTION STIMULI. We used a photo with added animation to provide realism and visual cues to judge familiar size and perspective of critical objects. Subjects viewed the picture of a woman holding a basket above the head, standing in front of a building (Movie S1). They fixated a dot on the basket. In active tasks, a ball moved upward from the basket with a randomized initial speed and a constant acceleration, bounced on the cornice of the building (4 m above the basket), and returned downward to the basket. Upon bouncing on the cornice, the ball velocity reversed sign without losing momentum, as would result from a coefficient of restitution equal to 1 (elastic bounce). The fixation dot expanded by 2-times for 200 ms after a random delay of 400 or 600 ms following the end of ball motion. Ball acceleration was either 1g (9.81 m s^{-2}) or -1g (-9.81 m s^{-2}). Initial speeds were chosen so as to obtain five uniformly distributed motion durations (1.4-1.78 s) for both 1g and -1g trials. We used a 2x2 factorial design crossing the type of accelerated visual motion (1g versus -1g) and the type of motor task (Proactive versus Reactive) in different blocks. In the Proactive task, subjects had to press a button with the right index finger so as to intercept the descending ball at the time of arrival at the fixation point. In the Reactive task, they had to press the button as fast as possible after the fixation dot expanded. During the baseline condition (No-motion), subjects had to simply fixate the dot expanding now without any ball motion. In each experiment, the five different blocks (1g Proactive, -1g Proactive, 1g Reactive, -1g Reactive and No-motion) were presented in sequence. A visual instruction informing about the upcoming task preceded Proactive, Reactive or No-motion trials, whereas no cue was given to identify either 1g or -1g trials that were presented without interruption in the sequence. The order of P/R, 1g/-1g blocks was permuted every three subsequent sequences, the starting sequence being counterbalanced across participants. A total of 12 such sequences were presented in each experiment. Within each block (lasting 25 s), each of the five different durations of ball motion was presented twice, in a randomized order. No feedback of response accuracy was provided.

Each subject performed the same protocol three times at a distance of three days between each session. The first two sessions were performed outside the scanner, and the third inside the scanner. We performed two sessions outside the scanner to train subjects intensively prior to scanning, and to verify motor performance under two different

conditions: the visual scene and the subject's head were positioned roughly vertical in the first session, whereas the visual scene and the subject's head were positioned roughly horizontal in the second session in the same orientation as in the session inside the scanner. In the first session, subjects sat at 60 cm in front of a 21"-PC-monitor and viewed a 38-cm wide picture (visual angle 38°). In the second and third session, subjects lay supine on the bed and viewed through a 45°-tilted mirror a 17-cm wide picture (visual angle 30°) rear-projected, using an LVP-X300U, Mitsubishi LCD projector (1280 x 960 pixels, 75-Hz refresh rate), onto a screen mounted on the head coil at 33-cm distance. Ball motion spanned 14°-visual angle. Button-press responses were recorded in all sessions by A/D sampling the voltage signal of the button at 1 KHz. Eye movements were recorded outside the scanner at 500 Hz with the EyeLink II (SR research, Mississauga, Ontario), 0.005° resolution. Average results reported in Fig. 1B were obtained inside the scanner (session 3), but they did not differ systematically from those obtained in the two previous sessions.

EXPERIMENT 2: DIRECT VESTIBULAR STIMULI. Caloric vestibular stimulation was performed by irrigating the ear with 60 ml of water at 10° C for 1 min (S1-S5). Water was injected by means of a syringe connected to a Y-shaped flexible plastic tube (4 mm outer diameter) inserted for about 1 cm in the external auditory canal of each ear. A valve allowed irrigating either the right or left ear. Water exiting from the ears was collected in plastic bags. Subjects were tested with the vestibular protocol twice. In the first session (outside the scanner), they lay supine on a bed in darkness and underwent one caloric test for each ear at 10 min interval. Eye movements were recorded to measure the parameters of the vestibular nystagmus. The second session was performed 7 days later in the scanner. Three caloric tests were performed for each ear, alternating between left and right ear, the starting side being counterbalanced across participants. Each fMRI session included 1 min baseline, followed by 1 min irrigation of one ear and 3 min post-stimulation period. An interval of 10 min between consecutive irrigations allowed for full recovery before the next vestibular stimulation. Subjects were blindfolded. They were interviewed after the scan about the subjective intensity of self-motion and other sensations induced by the vestibular stimuli. All reported moderate to strong vestibular sensations (illusory perception of tilt), but nobody reported pain, nausea, sweating, or emotional discomfort. In supine subjects, it is known that caloric irrigation elicits convective stimulation of horizontal semicircular canals, but results in central vestibular responses that are related to both virtual angular and gravito-inertial accelerations due to canal-otolith interactions (S6). Therefore, caloric stimuli are presumably adequate to activate central networks involved in processing both canal and otolith information (S6).

Scanning and data analysis.

Whole-brain fMRI data were acquired on a 1.5 T Magnetom Vision (Siemens) equipped with a quadrature head volume coil. Functional images were obtained with echo-planar T2* sequence using blood-oxygenation-level-dependent contrast, each comprising a brain volume of 37 interleaved axial slices (3-mm thick). Volumes were acquired with a repetition time of 4 s (echo time: 60 ms; field of view: 192 mm; matrix size: 64 x 64). In experiment 1 (visual motion stimuli), 408 volumes were acquired for each participant, divided in three sessions of 136 scans each. The total duration of the experiment was

approximately 29 min, with a 1-min pause every four sequences. In experiment 2 (direct vestibular stimuli), 450 volumes were acquired for each participant, divided in six sessions of 75 scans each.

Statistical parametric mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, UK) was used for image realignment (S7), normalization to Montreal Neurologic Institute (MNI) standard space, smoothing by a 6-mm FWHM Gaussian kernel, and statistical analysis (S8). We used a two-stage approach based on a stringent random-effects model performed on the group data (n=17, S9-S10).

In the first stage, the time series of each participant were high-pass filtered at 128 s and pre-whitened by means of an autoregressive model AR(1) (S8). All tasks were modelled as box-car functions convolved with the canonical hemodynamic response function (HRF). In experiment 1, we assessed the main effect of the type of target acceleration (1g trials minus -1g trials, and vice-versa), the main effect of the type of motor task (Proactive trials minus Reactive trials, and vice-versa), and any interaction between these two factors. In experiment 2, we compared brain activity during the first minute after the end of the ear-irrigation with the activity during the baseline (the 1-min period before irrigation). Note that this comparison excludes the somatosensory and auditory stimuli associated with irrigation, but it will capture vestibular responses, as it has previously been shown (S2-S3). Since we were interested in the overall pattern of brain activation following vestibular stimulation, rather than any brain-laterality issue related to unilateral ear-irrigations (S11), we averaged the data for the right and left ear-irrigations. The presence of artifacts due to the paramagnetic properties of water poured in the ear was excluded by means of careful inspection of the images. Moreover, only functional time series that showed a displacement of less than 2 mm before motion correction were included.

In the second stage of the analysis of both experiments, the effects obtained at single-subject level were used to compute one-sample t-tests assessing the significance of the effects of interest at the group-level (d.f.=16). The resulting maps were thresholded at P<0.05 corrected for multiple comparisons at cluster level (cluster size estimated at P<0.01), using distribution approximations from the theory of Gaussian fields (S12). Additional activations at lower thresholds are reported in the Tables when these were found at anatomical locations symmetrical to significant clusters in the opposite hemisphere. To statistically assess any common activation for the two primary effects of interest (i.e. the main effect of 1g minus -1g, and the main effect of vestibular stimulation), conjunction analyses were performed at the group-level (S13). We thresholded the resulting probability map by applying false discovery rate correction (FDR) (S14-S15). For this purpose, we set the allowed proportion of false positives (α) to 5%.

Supplementary results

Eye movements.
In experiment 1, subjects maintained fixation very well in all tasks. On average, subjects made 1.5±0.4 saccades (>1°-amplitude) per block, with no significant differences among the various conditions (two-factors ANOVA, P>0.6).
In experiment 2, subjects had normal, symmetrical responses to the standard caloric test and showed no signs of positional nystagmus when tested clinically. Caloric irrigation elicited slow-phase horizontal nystagmus directed toward the irrigated ear (peak velocity, 12.4±2.9°/s at 116 ± 14 s).

Supplementary Figure S1: Main effect of -1*g* visual motion in fMRI
Left: Activations in lateral occipital regions with -1*g* visual motion (main effect, [(-1*g*P) + (-1*g*R)] > [(1*g*P) + (1*g*R)]), overlaid on axial brain section. For display, the right side of the image corresponds to the right side of the brain. Right: Mean activity (± s.e.m., *n*=17) for the right cluster (347 voxels centred around the lateral occipital sulcus). For coordinates of peak activations see table S3.

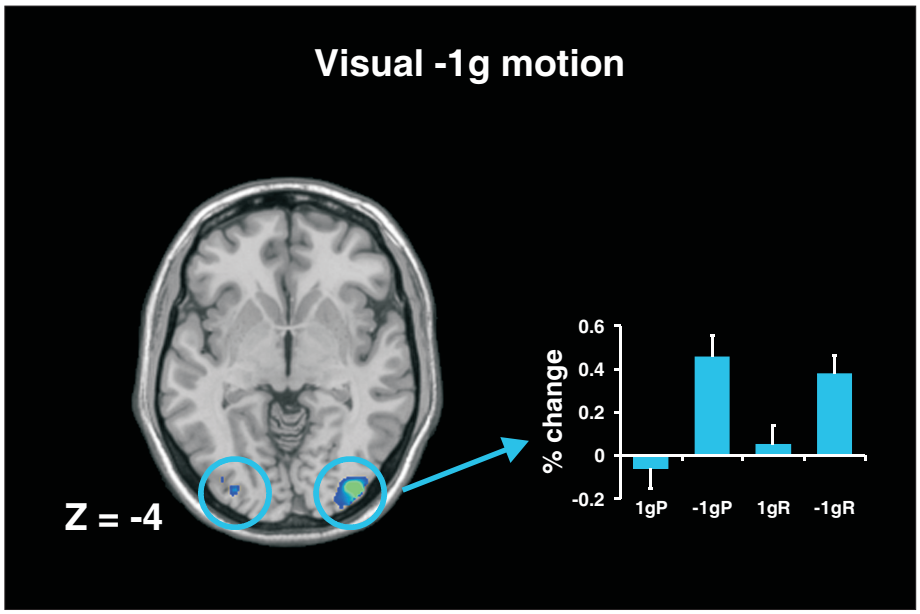

Fig. S1

Supporting tables.

Table S1. Cerebral foci of activation common to 1*g* visual motion and caloric vestibular stimulation.

		1*g* visual motion		Caloric vestibular		Common activations	
Brain region	**Side**	**x,y,z**	**Z-score**	**x,y,z**	**Z-score**	**x,y,z**	**Z-score**
Insula	L	-44, -6, 16	3.29	-44, -2, 8	3.68	-44, -2, 10	4.51
	L	-34, 0, 12	3.07	-34, 2, 10	2.74	-34, 2, 12	4.22
	L	-42, -4, -6	2.37	-40, 4, -8	3.75	-42, -4, -6	3.94
	R	36, -4, -8	2.96	40, 4, -8	4	36, -2, -8	4.63
Retroinsular cortex	L	-48, -36, 16	3.01	-46, -32, 18	3.61	-48, -34, 18	4.27
Supramarginal gyrus	R	62, -38, 32	3.88	60, -32, 42	5.33	62, -36, 32	5.97
Superior temporal gyrus	L	-56, -32, 14	(3.21)	-54, -36, 22	3.71	-58, -32, 16	4.38
	R	64, -38, 20	4.32	66, -36, 18	4.11	66, -36, 18	6.2
Precentral gyrus / Inferior frontal gyrus	L	-58, 12, 16	3.13	-58, 16, 4	2.85	-58, 16, 4	4.7
	R	62, 10, 14	(3.18)	54, 12, 10	4.36	62, 16, 14	5.00
Supplementary Motor Area	L	-6, -8, 62	3.94	-12 ,2, 72	(3.06)	-12, 2, 72	4.48
Cingulate cortex	L	-2, -10, 42	3.11	-4, -2 ,40	(3.00)	-8, 2, 46	4.39
	R	6, 0, 44	2.86	8, 16, 36	(3.17)	4, 0, 42	4.23
Postcentral gyrus	L	-30, -32, 62	3.23	-24, -42, 58	(2.81)	-38, -42, 60	4.17
	R	62, -20, 34	(2.74)	68, -16, 14	3.9	64, -20, 34	4.24
Thalamus	L	-14, -24, 2	3.16	-16, -18, -4	(2.74)	-16, -20, -2	3.99
Putamen	R	28, 4, -6	4.92	22, 6, -2	(2.3)	22, 6, -2	3.69

Note. Main effect of 1*g* visual motion across both Proactive and Reactive tasks ([(1*gP*) + (1*gR*)] > [(-1*gP*) + (-1*gR*)]; main effect of caloric vestibular stimulation ([left ear stimuli + right ear stimuli] > baseline); common activations between 1*g* visual motion and caloric vestibular stimulation determined from conjunction analysis. MNI coordinates (in mm) and *Z*-scores of anatomical foci showing peak activations. For 1*g*-motion and caloric stimulation, *Z*-scores were significant at $P<0.05$ corrected for multiple comparisons at cluster level (cluster size estimated at $P<0.01$), except for bracketed values that were significant at $P<0.01$ uncorrected. Common activations were determined by means of conjunction analysis with FDR at $\alpha = 0.05$.

Table S2. Cerebral foci of activation specific to either 1*g* visual motion or caloric vestibular stimulation.

		1*g* visual motion		Caloric vestibular	
Brain region	**Side**	**x,y,z**	**Z-score**	**x,y,z**	**Z-score**
Retroinsular cortex	L	-34, -38, 10	4.41	-52, -10, 10	4.2
	R			52, -16, 4	2.67
Supramarginal gyrus	L	-30, -44, 48	(3.2)	-62, -26, 42	(4.07)
Precentral gyrus / Inferior frontal gyrus	L	-58, 6, 20	3.92		
Supplementary Motor Area	R	12, 0, 66	3.27		
	L	-4, 2, 52	4.05		
Postcentral gyrus	L			-64, -18, 16	3.2
	R	32, -30, 60	3.24		
Middle frontal gyrus /Precentral gyrus	R			48, 3, 42	(4.01)
Superior occipital gyrus	L	-14, -102, 18	2.59		
	R	24, -98, 18	3.26		
Cerebellum	R	32, -54, -30	3.93		
	R	2, -62, -38	3.64		
Brainstem / Midbrain	L	-6, -24, -6	2.94		
	R	6, -32, -6	2.68		

Note. Main effect of 1*g* visual motion across both Proactive and Reactive tasks and main effect of caloric vestibular stimulation for regions activated for one of the two modalities only. Z-scores were significant at $P<0.05$ corrected for multiple comparisons at cluster level (cluster size estimated at $P<0.01$), except for bracketed values that were significant at $P<0.01$ uncorrected.

Table S3. Cerebral foci of activation with -1*g* visual motion.

Brain region	**Side**	**x,y,z**	**Z-score**
Middle / Inferior occipital gyrus	L	-28, -88, -4	(3.14)
	R	42, -88, -4	4.52

Note. Main effect of -1*g* visual motion across both Proactive and Reactive tasks ([(-1*gP*) + (-1*gR*)] > [(1*gP*) + (1*gR*)]. Z-scores were significant at $P<0.05$ corrected for multiple comparisons at cluster level (cluster size estimated at $P<0.01$), except for bracketed value that was significant at $P<0.01$ uncorrected.

Table S4. Cerebral foci of activation with Proactive task.

Brain region	**Side**	**x,y,z**	**Z-score**
Superior Frontal Gyrus	L	-22, 2, 58	5.54
	R	20, 0, 68	3.34
Precentral gyrus	L	-54, 0, 38	4.88
	R	30, -2, 50	4.48
Inferior frontal gyrus	L	-60, 14, 16	3.59
	R	50, 8, 12	3.38
Supplementary Motor Area	L	-10, -8, 54	3.00
	R	10, -18, 54	4.69
Middle cingulum	L	-12, -18, 40	3.48
	R	14, -34, 40	2.81
Superior parietal lobule	L	-22, -54, 52	5.09
	R	16, -60, 56	4.86
Inferior parietal lobule	L	-38, -42, 38	4.44
	R	38, -38, 46	4.31
Postcentral gyrus	L	-58, -18, 32	4.22
	R	40, -42, 66	3.88
Precuneus	L	-8, -48, 56	3.31
	R	14, -54, 60	4.04
Middle / Inferior temporal gyrus	L	-46, -58, -4	3.76
	L	-48, -46, 4	3.17
	R	44, -68, 10	4.64
	R	52, -64, -8	4.5
Fusiform gyrus	L	-40, -60, -18	3.04
Superior temporal gyrus	L	-60, -48, 22	2.94
Superior / Middle occipital gyrus	L	-24, -86, 34	4.67
	L	-48, -78, 8	4.98
	R	34, -78, 16	4.18
	R	26, -82, 18	4.13

Note. Main effect of Proactive motor task across both 1g and -1g stimuli ([(1gP) + (-1gP)] > [(1gR) + (-1gR)]). Z-scores were significant at P<0.05 corrected for multiple comparisons at cluster level (cluster size estimated at P<0.01). The opposite main effect (Reactive minus Proactive) did not show any significant activation.

Table S5. Cerebral foci of activation associated with interaction between type of visual acceleration and type of motor task.

Brain region	Side	x,y,z	Z-score
Cerebellum	L	-42, -64, -40	3.78
Precuneus	R	12, -38, 2	3.76
Posterior cingulum	R	6, -42, 14	3.31

Note. Interaction between 1*g* / -1*g* visual motion and Proactive / Reactive task: ([(1*gR*) - (-1*gR*)] > [(1*gP*) - (-1*gP*)]). Z-scores were significant at $P<0.05$ corrected for multiple comparisons at cluster level (cluster size estimated at $P<0.01$). The opposite interaction effect ([(1*gP*) - (-1*gP*)] > [(1*gR*) - (-1*gR*)]) did not show any significant activation.

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