in the incidence and distribution of neurons whose chemosensory mechanisms are temperature-sensitive. However, the absence of significant bitterness during warming and the reports of bitterness during cooling on circumvallate papillae raise the possibility that thermal sensitivity in some gustatory neurons may arise from cellular processes that are unrelated to chemosensory transduction.

We note that thermal taste was nearly discovered 35 years ago by von Békésy. In a well-known but controversial paper, von Békésy[15] reported that taste and thermal stimuli (heated or cooled water) presented to opposite sides of the tongue merged into a single sensation when warm water was paired with sucrose or quinine, or when cold water was paired with citric acid or NaCl. This observation led him to propose the 'Duplexity Theory of Taste'[15], in which he posited that "warm and cold stimuli act similarly to the four primary taste stimuli…" Our results now suggest that von Békésy's subjects may have reported a single sensation in the middle of the tongue when bilateral thermal and chemical stimuli evoked the same taste quality. □

Methods

Thermal taste screening procedure

The incidence of thermal taste was tested in naive subjects (8 males and 16 females, most of whom were students at Yale University) using three temperature conditions that pilot tests had shown were capable of producing sweetness, sourness and saltiness, respectively: warming from 20 to 35 °C, cooling from 35 to 15 °C, and cooling from 35 to 5 °C. Temperature was varied at approximately $\pm 1.5\,^{\circ}\mathrm{C\,s^{-1}}$ using an 8 mm × 8 mm computer-controlled Peltier thermode with thermocouple feedback. The thermode was affixed to a pencil-sized water-circulated heat sink and covered with plastic wrap for hygienic purposes. On each trial the thermode was set to the starting temperature, and with guidance from the experimenter and the aid of a mirror, subjects used the heat sink as a handle to position the thermode against the tongue. Heating or cooling began as soon as the temperature at the tongue–thermode interface stabilized at the starting temperature (5–10 s). Subjects were told to attend to the temperature change and to report if they perceived any other sensations, including tastes (defined as sweetness, sourness, saltiness or bitterness); they were assured that not everyone perceived such sensations, and that the purpose of the study was to discover how often and under what conditions they might appear. Stimulation began on the tongue tip and proceeded stepwise along the edge of the tongue to a distance ~5 cm caudal to the tip. Both sides of the tongue were tested, and each temperature condition was applied twice to each test site. When tastes were detected subjects reported their intensities verbally using a scale from 1 to 10. These ratings served to locate 'best' sites for thermal taste that were later tested more systematically.

Thermal testing on the tongue tip

The thermode was used to warm or cool the tongue tip over a series of temperature steps (ΔTs) that increased from 20 °C in steps (°C) of +5, +10, +15 and +20, or decreased from 35 °C in steps (°C) of −10, −15, −20, −25 and −30. Subjects rated the intensity of taste (sweetness, sourness, saltiness, bitterness) and thermal sensations (warmth, cold) using the labelled magnitude scale (LMS)[16], a continuous scale of sensation intensity bounded by 'no sensation' and 'strongest imaginable oral sensation'. The LMS was displayed on a computer monitor and subjects made their ratings using a mouse. Instructions were given to "attend now" as soon as heating began on warming trials and as soon as the target temperature was reached on cooling trials. Different instructions were used for heating and cooling because pilot tests had shown that sweetness occurred only while temperature rose, whereas sourness and saltiness persisted at steady temperatures. Because thermal taste was always accompanied by temperature sensations, taste and temperature ratings were obtained separately to help subjects make independent judgments. Each condition was presented twice in pseudo-random sequence.

Testing on 'best' thermal taste sites

18 subjects (one of the original 19 left the study between experiments) rated thermal tastes and temperature sensations in the same manner as on the tongue tip, except temperature was varied only as follows: from 20 to 35 °C to assess T_{SW}, from 35 to 15 °C to assess T_{SO}, and from 35 to 5 °C to assess T_{SA}. Chemical taste was assessed in a separate session on the same sites using four aqueous taste solutions (0.5 M sucrose, 0.1 M citric acid, 0.5 M NaCl and 0.01 M QHCl) found in pilot tests to produce approximately 'moderate' sweetness, saltiness, sourness or bitterness, respectively, when applied to small areas of the tongue. The experimenter used cotton-tipped applicators to carefully swab these solutions onto T_{SW} and T_{SO} 'best' sites for 3 s. Subjects used the LMS to rate intensity and rinsed between trials with distilled H_2O. Two replicates were obtained for each thermal and chemical condition.

Stepwise spatial testing

Measurements of T_{SW} and T_{SO} on the edge of the tongue were made on another group of 15 subjects (12 females and 3 males, screened as before from a sample of 22 females and 8 males). Seven sites were tested: the tongue tip and three contiguous locations on either side of the tip. Stimulation began at the tip and stepped approximately one width of the thermode (8 mm) at a time, first along one side of the tongue and then the other. At each site the thermode was warmed from 20 to 35 °C or cooled from 35 to 15 °C, with cooling and warming trials blocked. Two replicates were obtained for each temperature condition at each site.

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Postsaccadic visual references generate presaccadic compression of space

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With every rapid gaze shift (saccade), our eyes experience a different view of the world. Stable perception of visual space requires that points in the new image are associated with corresponding points in the previous image. The brain may use an extraretinal eye position signal to compensate for gaze changes[1,2], or, alternatively, exploit the image contents to determine associated locations[3,4]. Support for a uniform extraretinal signal comes from findings that the apparent position of objects briefly flashed around the time of a saccade is often shifted in the direction of the saccade[5–9]. This view is challenged, however, by observations that the magnitude[4,10] and direction[11] of the displacement varies across the visual field. Led by the observation that non-uniform displacements typically occurred in studies conducted in slightly illuminated rooms[4,7,10–13], here we determine

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the dependence of perisaccadic mislocalization on the availability of visual spatial references at various times around a saccade. We find that presaccadic compression[11] occurs only if visual references are available immediately after, rather than before or during, the saccade. Our findings indicate that the visual processes of transsaccadic spatial localization use mainly postsaccadic visual information.

We asked five observers to locate a briefly flashed (8-ms) luminous bar on a projection screen in a dark room while they were making saccades to the right. The bar was flashed at variable times before or after saccade onset. It appeared randomly at one of four locations around the saccade goal. Subjects reported the perceived location of the bar with a mouse pointer that appeared 500 ms after the saccade. A ruler that was projected on the screen provided visual references. The ruler could be switched on or off at various times during a trial. This allowed us to choose the times at which visual references were available. Figure 1 shows responses obtained from a subject when the ruler was available. Locations between the fixation point and the saccade goal are mislocalized in the direction of the saccade. Locations beyond the saccade goal are mislocalized against the direction of the saccade. The mislocalization starts about 70 ms before the saccade and continues during the saccade, until about 70 ms after saccade onset. We defined two index measures to compare the mislocalizations across subjects and conditions. The shift index describes the overall perceived shift in the direction of the saccade. It is defined as the mean over the four mean apparent positions of the bar. The compression index describes the strength of the compression. It is defined as the standard deviation of the four mean apparent positions. Both indices are normalized to their respective average values 100 ms before and after the saccade. Because the individual index curves were similar across subjects we averaged their data. Figure 2 shows the perisaccadic time course of these two measures when the ruler was present (blue curves). There is strong compression. In addition, there is also a shift in the mean apparent position. When we removed the ruler from the screen (that is, when no visual references were available) the compression was much weaker (Fig. 2, red curves). The shift is slightly larger in this case. This clearly shows that the pattern of perisaccadic mislocalizations is influenced by visual references. Without visual references, mislocalization is more uniform in direction and magnitude. This presumably reflects a unary extraretinal signal which is the only available information about the change of gaze direction in this case. When visual references are present, the metric of the mislocalization changes. All perceived positions move closer together and cluster around the saccade goal.

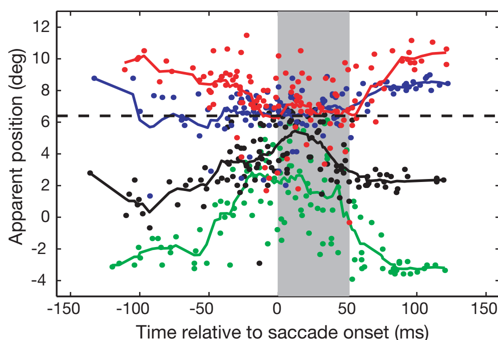

Figure 1 Perceived location of a flashed bar at position −2.6° (green), +2.6° (black), +10° (blue) and +13° (red) as a function of time relative to the onset of a saccade from −6.4° to +6.4°. Each point is a single measurement. Lines are running averages through the data, obtained with a gaussian filter of 33-ms standard deviation. Dashed line indicates the saccade goal. Around the time of the saccade, perceived locations are strongly biased towards the saccade goal.

What aspect of the visual references provided by the ruler induces this change? The ruler may serve as a stable transsaccadic reference frame that provides matching visual locations before and after the saccade. If this were true, one would predict less compression if the ruler was absent either before or after the saccade. It could also be that the ruler directly provides a reference frame for the flash. In this case one would expect less compression if the ruler was absent at the time of the flash. Finally, it might be that the visual motion of the ruler during the saccade is important to induce the compression. In this case one would expect less compression if the ruler was absent during the saccade, but not before or after it. To investigate these possibilities we ran a series of further experiments in which the ruler was switched on or off at different times during the trial. In each case we determined the mean presaccadic compression by averaging the compression index values of each subject between 50 and 0 ms before the saccade, and then took the mean across subjects. The results are expressed as percentages with 100% compression corresponding to the percept where all flashes are seen at the same place (Fig. 3).

We first tested whether the ruler acts as a stable transsaccadic reference frame. We presented the ruler selectively before or after the flash of the bar. In the pre-flash condition, the ruler was on at the beginning of the trial, stayed on until the presentation of the flashed bar and then went off with the bar. In the post-flash condition, the ruler was off initially and was switched on when the flashed bar appeared. We found very little compression in the pre-flash condition but strong compression in the post-flash condition (Fig. 3). We conclude that the compression does not rely on a stable transsaccadic reference. Rather, it must be induced by visual references present after the flash.

In two further experiments we tested whether the presence of the ruler during the saccade or immediately after the flash is important. In the gap condition, the ruler was on before and after the saccade but was switched off for 250 ms starting from the flash of the bar. Some compression occurred but it was not significantly different from that found when the ruler was absent either throughout the trial or after the flash (Fig. 3). In the post-saccade condition the

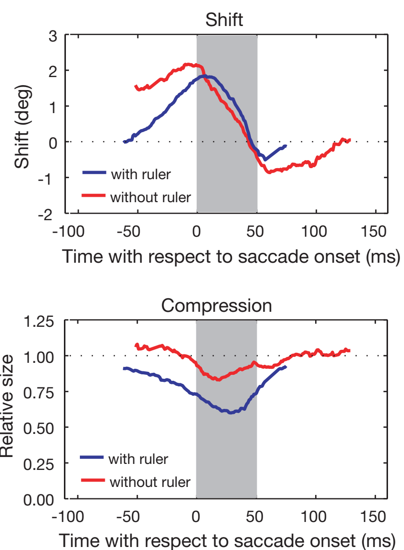

Figure 2 Perceived shift and compression as a function of time in the presence (blue) and absence (red) of visual references. Perceived shift is the mean of the four mean apparent positions of the bar relative to the mean apparent positions more than 100 ms before or after the saccade. Perceived compression ('relative size') is the standard deviation of the four mean apparent positions relative to the respective value more than 100 ms before or after the saccade. A value of 1 indicates no compression, a value of 0 would occur if all four positions appeared in a single place. Shift and compression were calculated from the individual localization curves of five subjects and then averaged.

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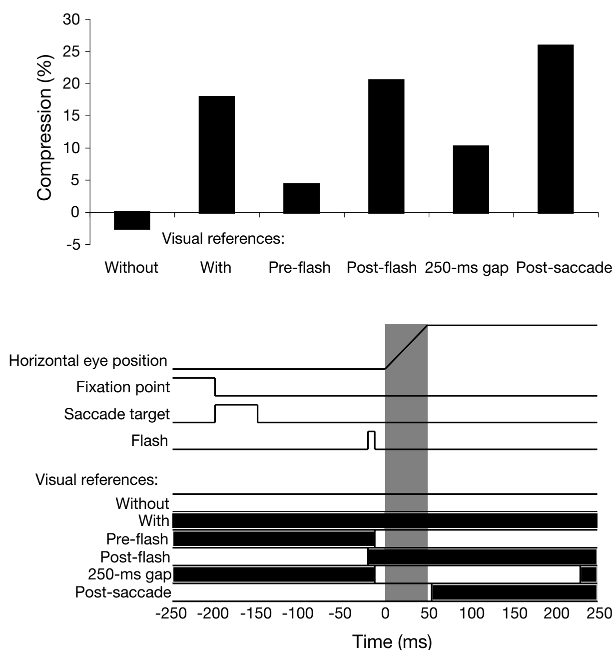

Figure 3 Magnitude of presaccadic compression for different presentation times of visual references. Top, height of bar is proportional to the mean of the compression measurements from –50 to 0 ms before the saccade, averaged across subjects. Compression in the with, post-flash and post-saccadic conditions is significantly different from that in the without and pre-flash conditions (analysis of variance and Student–Newman–Keuls post-hoc testing, $P < 0.05$). Bottom, timing of events in the different conditions. Black areas show the times when the ruler was present. The first two conditions (without/with visual references) are the same as in Fig. 2. In the pre-flash condition, visual references were only available before the flash. In the post-flash condition, visual references were initially absent but appeared with the flash and stayed thereafter. In the gap condition, visual references were turned off for 250 ms starting from the flash of the bar. In the post-saccadic condition, visual references appeared only after the saccade.

ruler was initially absent and switched on immediately after the saccade. We used the eye movement to trigger the presentation of the ruler. The stimulation program detected the start of the saccade and switched on the ruler 50 ms later, at the time when the saccade had just finished. This condition gave strong compression (Fig. 3). We conclude that visual references or visual motion during the saccade are not important. Rather, the presence of visual references immediately after the saccade elicits compression.

These results reconcile the seemingly disparate findings of refs 9, 10. In ref. 9, where the compression was described, continuous visual references were available, such as the visible frame of the monitor and the constantly present fixation and saccade targets. In ref. 10, which reported only shifts in the direction of the saccade, all potential visual references (the fixation point, saccade goal and comparison targets) were extinguished before the saccade.

Why then does the presence of postsaccadic visual references lead to a compression of presaccadic space, which is not seen in the absence of visual references? First, it is important to realize that an observer faces different problems in these two conditions[13]. To refer the presaccadic retinal signal to the appropriate world coordinates in the absence of visual references, the visual system can only subtract an eye-position signal. No sources of information beyond this signal are available, so any inaccuracies in this signal will be translated into misclocalizations[3,5–9]. In the presence of postsaccadic visual references, on the other hand, the task changes from egocentric localization to a relative position judgement in a retinal frame of reference, that is, with respect to other visible targets. We believe that different mechanisms are involved in these tasks[14]. Second, around the time of the saccade, mechanisms underlying perceptual stability are operating. Perceptual stability relies strongly on the visual information present immediately after the saccade finishes[15]. At that time, the postsaccadic location of the saccade target is determined from the visual scene[16]. In abstract terms, the internal presaccadic coordinate system is being remapped to comply with the postsaccadic eye position. Owing to the latency of visual processing and the anticipatory nature of remapping[17], a presaccadic stimulus could be interpreted in a visual coordinate system that is being remapped, or in one that has already reached its postsaccadic state. In either case a mislocalization will result. In this interpretation, the presaccadic compression of space is a signature of the fact that the visual remapping process is not instantaneous or uniform over the retina and that it requires early postsaccadic image information. □

Methods

Subjects sat in a dark room (luminance <0.1 cd m^{-2}) in front of a large projection screen. Visual stimuli were generated by a computer and presented on the projection screen by a video projector with a frame rate of 120 Hz. Each trial started with the appearance of a fixation dot 6.4° left of the screen centre followed after 1,700–1,870 ms by the appearance of a dot marking the saccade goal 6.4° right of the centre. The fixation point and saccade goal were extinguished 50 ms later (Fig. 3, lower panel). The subject was required to make a saccade from the fixation point to the saccade goal. Because saccadic latencies were between 110 and 190 ms, neither the fixation point nor the saccade target were visible at the time of the saccade. At a random time within 250 ms after the appearance of the saccade goal, a vertical bar (0.5° × 90°, mean luminance 20 cd m^{-2} was flashed at one of four possible positions (–2.6, 2.6, 10 or 13.6°) for one video frame (8 ms). About 500 ms after the saccade, a mouse pointer appeared. The subject moved the mouse pointer to the apparent horizontal location of the bar and pressed a button. This location was recorded along with the time of the flash. Then the next trial started.

Visual references were provided by a horizontal ruler displayed on the screen. The ruler was a horizontal white line (luminance 20 cd m^{-2}) with short vertical lines at 12.8° intervals, each labelled with a number. One of these marks fell on the fixation point, another on the saccade goal. The ruler extended over the entire width of the screen. In the first experiment, the ruler was continuously visible. In the second experiment it was completely absent. In the remaining four experiments the ruler was switched on or off at different times during the trial. The lower panel of Fig. 3 shows the timing of events in the different conditions. All subjects performed the complete set of experiments. Between 170 and 300 responses were collected per subject and condition.

Eye movements were measured with an Ober 2 infrared eye tracker at a sample rate of 200 Hz. Saccade initiation time was determined by a velocity criterion with a threshold of 10% of the maximum speed during the saccade. Every saccade was visually checked by the experimenter for appropriate direction, amplitude and timing. Trials in which the saccade did not meet the requirements of the task were discarded. The last experiment used additional electro-oculography to trigger the appearance of the ruler with the eye movement.

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A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*

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The tumour-suppressor gene *Neurofibromatosis 1* (*Nf1*) encodes a Ras-specific GTPase activating protein (Ras-GAP)[1–5]. In addition to being involved in tumour formation[6,7], *NF1* has been reported to cause learning defects in humans[8–10] and *Nf1* knockout mice[11]. However, it remains to be determined whether the observed learning defect is secondary to abnormal development. The *Drosophila* NF1 protein is highly conserved, showing 60% identity of its 2,803 amino acids with human NF1 (ref. 12). Previous studies have suggested that *Drosophila* NF1 acts not only as a Ras-GAP but also as a possible regulator of the cAMP pathway that involves the *rutabaga* (*rut*)-encoded adenylyl cyclase[13]. Because *rut* was isolated as a learning and short-term memory mutant[14,15], we have pursued the hypothesis that NF1 may affect learning through its control of the Rut-adenylyl cyclase/cAMP pathway. Here we show that NF1 affects learning and short-term memory independently of its developmental effects. We show that G-protein-activated adenylyl cyclase activity consists of NF1-independent and NF1-dependent components, and that the mechanism of the NF1-dependent activation of the Rut-adenylyl cyclase pathway is essential for mediating *Drosophila* learning and memory.

We examined olfactory associative learning of adult fruit flies by using a well-defined Pavlovian procedure[16–19]. Significant decrements in olfactory learning performance were shown for two independently isolated *NF1* null alleles[12], $NF1^{P1}$ and $NF1^{P2}$, as compared with K33, the parental line for *NF1* mutants with a P-element inserted nearby the *NF1* locus[12] (Table 1, Fig. 1a). Olfactory avoidance and electric-shock reactivity[20], two sensorimotor activities necessary for performing the learning task, were similar in the mutant and control K33 flies (Table 1). To consider the potential effects of genetic background on behaviour[20], we outcrossed *NF1* mutants and K33 with an isogenic line w^{1118} (*isoCJ1*)[21]. Again, learning scores of *NF1* mutants were significantly reduced (Table 1, Fig. 1a), whereas the parameters of sensorimotor activities were not statistically different from the control with a similar genetic background (Table 1). Even though learning scores and some scores for shock reactivity and odour avoidance are significantly different for K33 in different genetic backgrounds, these behavioural parameters also vary accordingly in *NF1* mutants (Table 1). These results indicate that *NF1* is a learning mutant.

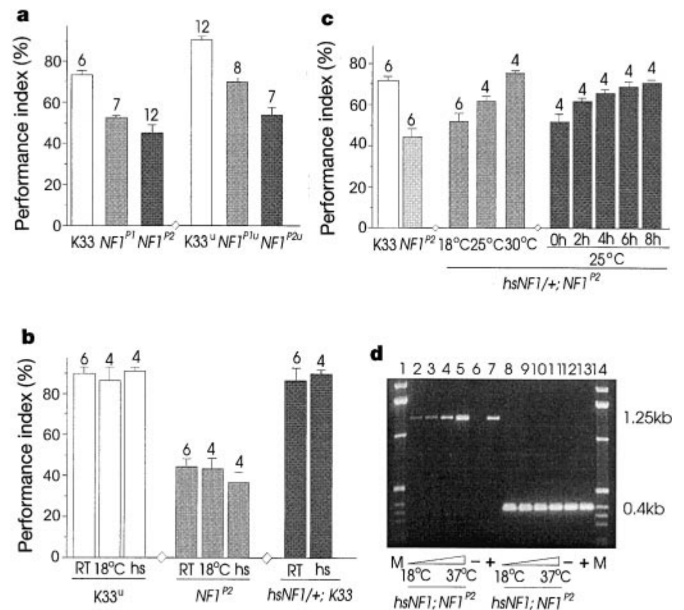

Figure 1 Rescue of the *NF1* learning defect by inducible expression of the normal *NF1* transgene. **a**, NF1 learning defects observed in both the original and outcrossed isogenic (marked by u in superscript) genetic background. K33 is the parental line of *NF1* mutants. **b**, No effect on learning scores for the heat-shock treatment in controls, including overexpression of the *NF1* transgene in the control background. **c**, Rescue of the learning defect by induced expression of the *NF1* transgene. In the first group, the flies were moved from 18 °C to 25 or 30 °C for 2 h before the learning test ($P < 0.05$, Tukey Kramer Honestly Significant Difference). In the second group, flies were shifted from 18 °C to 25 °C for 0, 2, 4, 6 or 8 h, respectively (significant for 2 h, $P < 0.05$). The number of assays for each group are indicated above each error bar. **d**, Semi-quantitative RT–PCR showing induced expression of the hsNF1 transgene. Lanes 1 and 14, 1-kb DNA ladder (M) (Gibco BRL). Lanes 2–7, RT–PCR using NF1-specific primers with cDNA prepared from *hsNF1*; $NF1^{P2}$ flies grown at 18, 25 and 30 °C or given daily 1 h heat shock at 37 °C, or from $NF1^{P1}$ mutant (–) flies or K33 wild-type (+) flies grown at 18 °C. Lanes 8–13, control RT–PCR from the same cDNA using ribosomal protein rp49-specific primers. Three separate mRNA isolations showed the same pattern of increased expression of the hsNF1 transgene at increased temperature.

Table 1 Performance indice for olfactor learning, shock reactivity and odour avoidance

Genotypes	Learning (*n*)	Shock reactivity		Odour avoidance			
				BA dilution		MCH dilution	
		60 V	20 V	4%	0.4%	Undiluted	10%
K33	73 ± 2 (6)	72 ± 6	25 ± 9	78 ± 5	28 ± 10	73 ± 7	40 ± 9
$NF1^{P1}$	53 ± 1 (7)*	77 ± 4	30 ± 5	80 ± 3	25 ± 4	66 ± 8	36 ± 6
$NF1^{P2}$	45 ± 4 (12)*	76 ± 3	26 ± 5	71 ± 4	19 ± 6	67 ± 4	29 ± 7
hsNF1/+; $NF1^{P2}$	75 ± 2 (4)	65 ± 3	23 ± 6	79 ± 6	32 ± 11	83 ± 4	34 ± 7
$K33^u$	90 ± 1 (12)	89 ± 2	61 ± 6	92 ± 1	41 ± 8	77 ± 5	63 ± 5
$NF1^{P1u}$	70 ± 2 (8)	80 ± 5	56 ± 8	85 ± 5	36 ± 9	84 ± 6	59 ± 8
$NF1^{P2u}$	54 ± 4 (7)*	83 ± 3	62 ± 7	93 ± 2	41 ± 7	77 ± 4	58 ± 5

K33, $NF1^{P1}$, $NF1^{P2}$ and *hsNF1/+*; $NF1^{P2}$ have a similar genetic background, whereas $K33^u$, $NF1^{P1u}$ and $NF1^{P2u}$ have a different background (see Methods). All scores a e expressed as PI ± s.e.m. For learning, the number (*n*) of assays are indicated in parentheses. For all shock reactivity and odour avoidance assays, $n = 8$.
* Statistically different from control. No statistical difference at the level of $\alpha = 0.05$ is detected among all the sensorimotor activities. Learning defect is significant at $\alpha \leq 0.001$. Comparison is made between mutants and controls with a similar genetic background using Tukey–Kramer HSD test within the Macintosh software package JMP3.1 (SAS institute, Inc., Cary, North Carolina, USA).

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