chromosome effect: for both dependent variables at both times, XX differed from XY⁻ and XX*Sry* differed from XY⁻*Sry* (P < 0.02). Planned comparisons also revealed weaker sex effects: at 6 d.i.v., XY-Sry cultures had more THir neurons than XY- cultures (THir, P < 0.01, TH/MAP2, P < 0.05), but XX cultures had more THir neurons than XXSry cultures (TH/MAP2, P < 0.02). At 11 d.i.v., XY-Sry cultures had more THir neurons than XY- cultures (P < 0.02), but no significant differences between sexes were found in other groups or in the TH/MAP2 ratio.

The sex chromosome effect was robust, replicated using different embryos at two different times in vitro. The XX/XY- difference cannot be attributed to an effect of the Sry gene, which did not vary among the groups compared: Sry was absent in comparisons of XX and XY⁻ but present in comparisons of XXSry and XY-Sry. Rather, the sex chromosome effect can be attributed to differences in (i) expression of Y genes that are normally present only in male cells, (ii) differences in expression of X genes, especially those that escape X inactivation and/or (iii) differences in paternal imprinting of X genes, which occurs in some XX but not in XY cells^{1,11}. The sex chromosome effect was specific to THir neurons because it was seen in the TH/MAP2 ratio, and groups did not differ in number of MAP2ir neurons. The sex differences (XX versus XXSry; XY- versus XY-Sry) could be due to either a differential effect of gonadal secretions before cell harvesting, or the expression of Sry in male but not female brain cells¹². Most of the sex differences reflect a higher number of dopamine cells when Sry was present ($XY^-Sry > XY^-$), but in a single case (TH/MAP at 6 d.i.v.), the sex effect was paradoxically in the opposite direction (XX > XXSry).

The present results draw further attention to mesencephalic dopamine systems as potential sites of direct X or Y gene action during sexual differentiation of the brain, and raise the question of the site and mechanism of action of the sex chromosomes in neuronal development in vivo. The nigrostriatal and mesolimbic dopamine systems show pervasive sex differences in rodents and humans^{3,13,14}. Thus, we also question whether sex chromosome genes lead to functional differences and influence these neurons' susceptibility to disease. The in vitro system described here offers significant advantages for unraveling the cellular and molecular mechanisms of sexchromosome gene action.

Note: Supplementary information is available on the Nature Neuroscience website.

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- Arnold, A. P. in *Hormones, Brain and Behavior* (eds. Pfaff, D. W. et al.) 105–135 (Academic Press, New York, 2002).
- Reisert, I. & Pilgrim, C. Trends Neurosci. 14, 467-473 (1991).
- Pilgrim, C. & Reisert, I. Horm. Metab. Res. 24, 353-359 (1992).
- Weisz, J. & Ward, I. L. Endocrinology 106, 306-316 (1980).
- Picon, R. J. Endocrinology 71, 231–238 (1976). Sibug, R. et al. Devel. Brain Res. 93, 136–142 (1996). 5
- Beyer, C., Eusterschulte, B., Pilgrim, C. & Reisert, I. Cell Tissue Res. 270, 7. 547-552 (1992)

- S. Lovell-Badge, R. & Robertson, E. Development 109, 635–646 (1990).
 Mahadevaiah, S. K. et al. Hum. Mol. Genet. 7, 715–727 (1998).
 De Vries, G.J. et al. J. Neurosci. (in press).
 Xu, J., Burgoyne, P. S., & Arnold, A. P. Hum. Mol. Genet. 11, 1409–1419 (2002)
- 12. Lahr, G. et al. Mol. Brain Res. 33, 179-182 (1995).
- 13. Becker, J. B. Pharmacol. Biochem. Behav. 64, 803-812 (1999).
- 14. Dluzen, D. E. & McDermott, J. L. J. Gend. Specif. Med. 3, 36-42 (2000).

Auditory midbrain neurons that count

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Many acoustic communication signals, including human speech and music, consist of a precise temporal arrangement of discrete elements, but it is unclear whether this precise temporal patterning is required to activate the sensory neurons that mediate signal recognition. In a variety of systems, neurons respond selectively when two¹⁻³ or more⁴ sound elements are presented in a particular temporal order and the precise relative timing of these elements is particularly important for 'delay-tuned' neurons², including 'tracking' types⁵, in bats. Here we show that one class of auditory neurons in the midbrain of anurans (frogs and toads) responds only to a series of specific interpulse intervals (IPIs); in the most selective cases, a single interval that is slightly longer or shorter than the requisite interval can reset this interval-counting process.

Many anuran vocalizations consist of a series of pulses, repeated at specific and constant intervals⁶ (Fig. 1a). This temporal patterning of pulses is required for call recognition^{6,7}, and female anurans are able to differentiate between calls that differ in pulse number⁸. 'Pulse-integrating' neurons in the midbrain may be the neural substrate for this selectivity, as they show sharp tuning to pulse repetition rate (PRR)^{9,10}. We found that they also seem to require a threshold number of pulses to fire (Fig. 1b), and respond to nearly every presentation of a stimulus that has just one more than the threshold number of pulses (Fig. 1c).

Do these cells require a certain number of correct IPIs (interval-counting hypothesis), or a certain average pulse rate (number of pulses in an integration time window)?

We tested the interval-counting hypothesis by recording extracellularly from 33 pulse-integrating cells while presenting stimuli that differed in their pulse-interval distributions (data from a representative cell in Fig. 2a-c). This neuron responded when five or more pulses were presented at a rate of 100 pulses/s; there was a strong, tonic response when 10 pulses were presented with 10-ms IPIs (Fig. 2b). Across neurons, responses to the constant-interval stimulus ranged from phasic to tonic. No responses were seen, however, to a mixedinterval stimulus (Fig. 2c), even though it had the same average pulse rate as the constant-interval stimulus. That is, it consisted of 10 pulses in 100 ms. Repeated IPIs of 5 ms (the shorter



IPIs in the mixed-interval stimulus) were effective, although not optimal, in exciting this particular neuron (Fig. 2d); the mixed-interval stimulus, therefore, was not simply a composite of ineffective IPIs. Most neurons did not respond to the mixed-interval stimulus (Fig. 2e). Those that did respond weakly to this stimulus also responded when just 2–3 pulses were delivered at the unit's optimal rate.

To further test the interval-counting hypothesis, we placed a single long interval within a series of optimal IPIs (Fig. 3a, compare top and middle histograms). The exemplar shown required eight consecutive optimal intervals for response. An interval of just 30 ms completely reset the integration process; after this long interval, eight additional optimal intervals were needed to elicit responses (Fig. 3a, bottom). This 30-ms interval was similar to that seen in the 'encounter' calls of this species (Fig. 1a).

We defined the reset time as the minimum duration of the long interval that prevented the excitation from the first series of pulses from 'carrying over' to that from the second series of pulses (Fig. 3b). These two neurons had similar reset times (approximately 25 ms), but different integration times (41.5 ms versus 130 ms; for example, thirteen intervals of 10 ms each gives an integration time of 130 ms). Across cells, there was no significant relation ($r^2 = 0.002$, P = 0.874) between neural integration times and reset times (Fig. 3c). Reset times ranged from 15 to 40 ms (median, 30 ms).

Are these cells counting the number of consecutive intervals that are shorter than a particular duration, or counting those of a specific duration? Some neurons' response weakened only slightly as the PRR was increased above their optimal rates. These cells had a rather broad 'interval tolerance' and the integration process was not reset by a short interval. Neurons at the other extreme had very sharp PRR tuning and only responded when a threshold number of intervals of a specific

Fig. 2. Responses to constant-interval and mixed-interval stimuli. Histograms of the cumulative responses of a single neuron to seven repetitions of (\mathbf{a}, \mathbf{b}) 3 pulses and 10 pulses (10-ms |PI), (\mathbf{c}) interval between successive pulses alternated between 5 and 15 ms and (d) 5-ms intervals. Stimulus traces shown below histograms. Carrier frequency, 1,100 Hz. (e) Response levels of nine neurons in *H. regilla* (closed triangles) and ten neurons in *R. pipiens* (open circles) to constant-interval stimuli.

Fig. 1. (a) Oscillograms of calls from *Hyla regilla* and *Rana pipiens*. In *H. regilla*, sound pulses are repeated at a faster rate in the advertisement calls than in the aggressive call; for *R. pipiens*, this relation is reversed. Pulse-integrating cells in both species were tuned primarily to fast PRRs. (b) Raster plots and histograms of a cell's responses to 3 (upper) or 4 (lower) pulses repeated at a rate of 100 pulses/s. Three, or fewer, pulses were ineffective, but 4 pulses elicited spikes on 15 of 18 stimulus presentations. (c) Probability of response to each repetition versus the number of pulses in the stimulus for six cells. Recordings were from single neurons in the torus semicircularis of 12 *R. pipiens* and 5 *H. regilla*^{9,10}. Acoustic stimuli were presented once every 2.5 s from a speaker situated 0.5 meters from the frog, contralateral to the recording site.

value had occurred (Fig. 3d). For these 'narrow-band' cells, a single interval that was either too short or too long reset the integration process. In the most sensitive neuron (Fig. 3d, filled circles), the integration process was reset when the experimental interval differed from the optimal interval by only ~2 ms. Thus we conclude that these neurons were 'counting' the number of consecutive IPIs that fell within a certain tolerance.

The mechanisms underlying this interval-counting process are currently unknown, but preliminary simulations indicate that classic temporal summation can be ruled out. Some forms of synaptic facilitation, however, may be tenable¹¹.

Neurons have been recorded in other systems that show a strongly facilitated response when two, or in some cases more, temporally discrete signals are presented in a specific order. Delay-tuned cortical cells in bats require two FM sweeps, separated by a rather specific time interval, for maximal response^{2,5}. In songbirds, however, the precise timing between sound elements seems to be less critical for response⁴; this tolerance may relate to correct perceptual categorization across variation due to motivational state. The requirement shown here of neurons for a series of specific IPIs may be related to the regularity of IPIs in anuran communication signals. Finally, interval-counting processes may be important in human audition. In intervaldiscrimination tasks, subjects are best able to detect a change in interpulse (tone pip) interval when a series of intervals of each type are presented; when only one different interval is embedded in a series of identical intervals, subjects are less likely to detect the difference¹². Such results might be expected if interval-



Fig. 3. Effects of longer or shorter intervals in resetting the integration process. (a) Response histograms for a single neuron in H. regilla to a sequence of 9 pulses (top), two sequences of 8 pulses (middle) and sequences of 8 and 9 pulses (bottom). Carrier frequency, 600 Hz. Within each group, adjacent pulses were separated by 10 ms. The gap between pulse sequences was 20 ms. (b) Normalized response levels of two neurons versus the duration of the experimental interval (Int) that separated the two sequences of pulses. The number of intervals that preceded and followed the long interval was one less than threshold. Integration times were 41.5 ms (circles) and 130 ms (triangles). In both cases, the integration process was reset by a long interval of approximately 25 ms. Carrier frequency, 600 Hz (circles) or 2200 Hz (triangles). The optimal PRRs for each cell were 100 Hz (circles) and 120 Hz (triangles). (c) Integration times versus reset times for seven neurons from H. regilla and seven neurons from R. pipiens. Arrows point to data from the two neurons shown in (b). (d) Normalized response of two tightly tuned neurons versus the duration of the experimental interval. The number of intervals before and after the experimental interval in each cell was one less than threshold (circles) or equal to threshold (triangles; note the non-zero response).

tuned neurons exist in humans and, like in anurans, generally require several intervals to respond.

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Enhanced tactile spatial acuity and cortical processing during acute hand deafferentation

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Acute deafferentation of a limb results in bilateral cortical reorganization^{1,2}, but the behavioral consequences of this phenomenon are unknown. Here we found rapid improvements in tactile spatial acuity and changes in cortical processing for the left hand



- Margoliash, D. J. Neurosci. 3, 1039-1057 (1983). 1.
- Suga, N. J. Exp. Biol. 146, 277-286 (1989). 2.
- Wollberg, Z. & Newman, J. D. Science 175, 212-214 (1972). 3.
- 4.
- 5.
- Margoliash, D. & Fortune, E. S. J. Neurosci. 12, 4309–4326 (1992). O'Neill, W. E. & Suga, N. Science 203, 69–72 (1978). Gerhardt, H. C. in The Evolution of the Amphibian Auditory System. (eds. 6. Fritzsch, B. et al.) 455-484 (Wiley, New York, 1988).
- 7. Rose, G. J. & Brenowitz, E. A. Anim. Behav. 63, 1183-1190 (2002).
- Klump, G. M. & Gerhardt, H. C. Nature 326, 286-288 (1987). 8.
- Alder, T. B. & Rose, G. J. Nat. Neurosci. 1, 519-523 (1998). 9
- 10. Alder, T. B. & Rose, G. J. J. Comp. Physiol. 186, 923-937 (2000).
- 11. Fortune, E. S. & Rose, G. J. J. Neurosci. 20, 7122 (2000).
- 12. Keele, S. W., Nicoletti, R., Ivry, R. I. & Pokorny R. A. Psychol. Res. 50, 251–256 (1989).

during cutaneous anesthesia of the right hand. The site-specific improvement in tactile spatial acuity may represent a behavioral compensatory gain.

Twenty-four naïve subjects (18–48 years old, 13 females) participated in the experiments. All gave written informed consent, and the Institutional Review Board approved the study protocol. The limits of tactile spatial resolution were measured using Tactile Acuity Gratings (MedCore, www.med-core.com) in a grating orientation task (GOT; see Supplementary Methods and Supplementary Fig. 1 online). The GOT yields reliable and reproducible measures of spatial acuity³ that depend on the spatial response profile of slowly adapting afferents⁴. GOT thresholds were measured at the left index fingertip or lower lip immediately before (baseline), during (late into anesthesia) and 15 minutes after (post) recovery from cutaneous anesthesia of the right hand or foot (control condition) elicited by ischemic nerve block. Cutaneous anesthesia was determined by applying Semmes-Weinstein nylon monofilaments to the distal pad of the second finger or toe on the right. Cutaneous anesthesia was defined as the loss of touch detection during application of a filament exerting a force of 4 N.

Baseline GOT thresholds at the finger (1.1 \pm 0.09 mm) and lip $(0.78 \pm 0.07 \text{ mm}; T = 2.7, P = 0.012)$ were comparable to previous findings³. Right hand anesthesia resulted in better GOT performance at the left index finger in 17 of 19 subjects, averaging $18.0 \pm 3.7\%$ (from mean baseline threshold of 1.1 ± 0.09 mm to mean threshold of 0.89 ± 0.07 mm during