of a motion signal[22]. DeAngelis *et al.* conceive their cells as having receptive fields with corresponding positions of their envelopes in the two eyes but with a spatial phase difference. Their model can be generalized to include the possibility of temporal phase differences as well, or any combination of spatial and temporal phase difference, resulting in cells with phase differences in space–time. If such cells are repetitively stimulated at temporal frequencies near to their resolution limit, information about temporal phase will be lost, leading to the effects we have observed in our experiment. □

Received 5 June; accepted 13 September 1995.

1. Wheatstone, C. *Phil. Trans. R. Soc.* **2,** 371–393 (1838).
2. Barlow, H. B., Blakemore, C. & Pettigrew, J. D. *J. Physiol.* **193,** 327–342 (1967).
3. DeAngelis, G. C., Ohzawa, I. & Freeman, R. D. *Nature* **352,** 152–159 (1991).
4. Regan, D. & Beverley, K. I. *Vision Res.* **13,** 2369–2379 (1973).
5. Westheimer, G. & McKee, S. P. *J. opt. Soc. Am.* **68,** 450–455 (1978).
6. Morgan, M. J., Watt, R. J. & McKee, S. P. *Vision Res.* **23,** 541–546 (1983).
7. Anderson, S. J. & Burr, D. C. *Vision Res.* **25,** 1147–1154 (1985).
8. Burr, D. C. & Ross, J. *Vision Res.* **18,** 523–532 (1978).
9. Palmer, A. R., Rees, A. & Caird, D. *Hearing Res.* **50,** 71–86 (1990).
10. Pulfrich, C. *Naturwissenschaft* **10,** 553–564 (1922).
11. Ross, J. *Nature* **248,** 363–364 (1974).
12. Morgan, M. J. & Thompson, P. *Perception* **4,** 3–18 (1975).
13. Schor, C. M., Wood, I. C. & Ogawa, J. *Vision Res.* **24,** 573–578 (1984).
14. Derrington, A. M. & Lennie, P. *J. Physiol. Lond.* **357,** 219–240 (1984).
15. Livingstone, M. S. & Hubel, D. H. *Science* **240,** 740–749 (1988).
16. Morgan, M. J. & Watt, R. J. *Vision Res.* **22,** 863–866 (1982).
17. Morgan, M. J. & Tyler, C. W. *Proc. R. Soc.* B (in the press).
18. Ross, J. & Burr, D. in *Vision, Brain & Cooperative Processes* (eds Arbib, M. & Hanson, A. R.) (Bradford, Amherst, MA, 1983).
19. Burr, D. C., Ross, J. & Morrone, M. C. *Proc. R. Soc. Lond.* B**227,** 249–265 (1986).
20. Reid, R. C., Soodak, R. E. & Shapley, R. M. *J. Neurophysiol.* **66,** 505–529 (1991).
21. McLean, J., Raab, S. & Palmer, L. A. *Visual Neurosci.* **11,** 271–294 (1994).
22. Morgan, M. J. *Perception* **9,** 161–174 (1980).
23. Watt, R. J. & Andrews, D. P. *Curr. Psychol. Rev.* **1,** 205–214 (1981).

ACKNOWLEDGEMENTS. We thank L. Partridge and J. Levitt for helpful comments. Supported by a grant from the BBSRC.

Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase

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In addition to its role in blood vessel[1,2] and macrophage[3,4] function, nitric oxide (NO) is a neurotransmitter[5] found in high densities in emotion-regulating brain regions[6–8]. Mice with targeted disruption of neuronal NO synthase (nNOS) display grossly normal appearance, locomotor activity, breeding[9], long-term potentiation[10] and long-term depression[11]. The nNOS$^-$ mice are resistant to neural stroke damage following middle cerebral artery ligation[12]. Although CO_2-induced cerebral vasodilatation in wild-type mice is NO-dependent, in nNOS$^-$ mice this vasodilation is unaffected by NOS inhibitors[13]. Establishing a behavioural role for NO has, until now, not been feasible, as NOS inhibitor drugs can only be administered acutely and because their pronounced effects on blood pressure and other body functions obfuscate behavioural interpretations. We now report a large increase in aggressive behaviour and excess, inappropriate sexual behaviour in nNOS$^-$ mice.

In establishing our breeding colony of nNOS$^-$ mice, we housed groups of five nNOS$^-$ male mice together in cages and upon routine morning examinations often discovered one or two dead mice in each cage. Initial direct observations indicate that the male nNOS$^-$ mice engage in chronic aggressive behaviour, not apparent among nNOS$^-$ female mice or wild-type male or female mice housed together (Fig. 1).

To examine this behaviour in greater detail, we conducted studies of inter-male aggression in an intruder–resident model[14]. The following aggressive behaviours were scored: offensive attack, biting, wrestling and chasing. Tail rattling was not recorded as an aggressive behaviour. Submissive postures were also recorded. Submission was operationally defined as rolling onto the back with the paws extended. When wild-type mice are introduced to a cage, the latency to the first aggressive attack by the resident mice is the same for wild-type and nNOS$^-$ residents (Fig. 2). However, the nNOS$^-$ residents display 3–4 times more aggressive encounters than wild-type residents. Moreover, in these encounters attacks are initiated by the nNOS$^-$ residents in 87% of their encounters with the wild-type intruders, nearly six times higher than the proportion of attacks initiated by wild-type residents.

In another model we observed groups of four wild-type or nNOS$^-$ males together in an aquarium (Fig. 3). In this model the latency to first attack is less than 1 min for the nNOS$^-$ animals, about 1/5th the latency for wild-type mice. The nNOS$^-$ mice display almost twice as many attacks as wild-type animals during a 15 min observation period. It is likely that the disparity between the nNOS$^-$ and wild-type mice would be greater with a longer duration, but we stopped the encounters after 15 min to prevent serious wounding. The duration of aggressive encounters is also greater in nNOS$^-$ than wild-type mice. The most dramatic difference between the two groups involves the number of submissive postures. nNOS$^-$ mice display submissive postures only one-tenth as frequently as wild-type mice.

Inappropriate aggressiveness has only been observed in male nNOS$^-$ mice. In multiple experiments female nNOS$^-$ mice have not exhibited aggressive behaviour when challenged by an intruder female. Furthermore, female nNOS$^-$ mice grouped 4 per cage also do not display any aggressive behaviours.

Our initial behavioural observations suggested that the persistence of excessive behaviours seen in aggressive encounters extends to reproductive interactions. In our efforts to obtain timed pregnant females, we paired nNOS$^-$ males with nNOS$^-$ females at varying stages of oestrus. The males display excessive and inappropriate mounting behaviour associated with substantial vocal protestations by the females. To quantify this behaviour we conducted studies in which anoestrous females were paired with wild-type or nNOS$^-$ males commencing at 10:00. Animals were observed for 8 h and their behaviours were recorded by videotape and scored blindly. Two 'live' observers, also uninformed about the phenotype of the mice, scored the behavioural interaction during the first 15 min of each hour (Fig. 4). During the initial 15 min observation period wild-type and nNOS$^-$ males mount females to the same extent, reflecting the normal male response to a new female. This behaviour diminishes rapidly in wild types so that by 1 h after introduction into the cage the number of mounts by wild-type males decreases 50%. By contrast, only a modest, non-significant decrease in the number of mounts occurs for the nNOS$^-$ animals. By 7 and 8 h following introduction to the cage, wild-type mouse encounters

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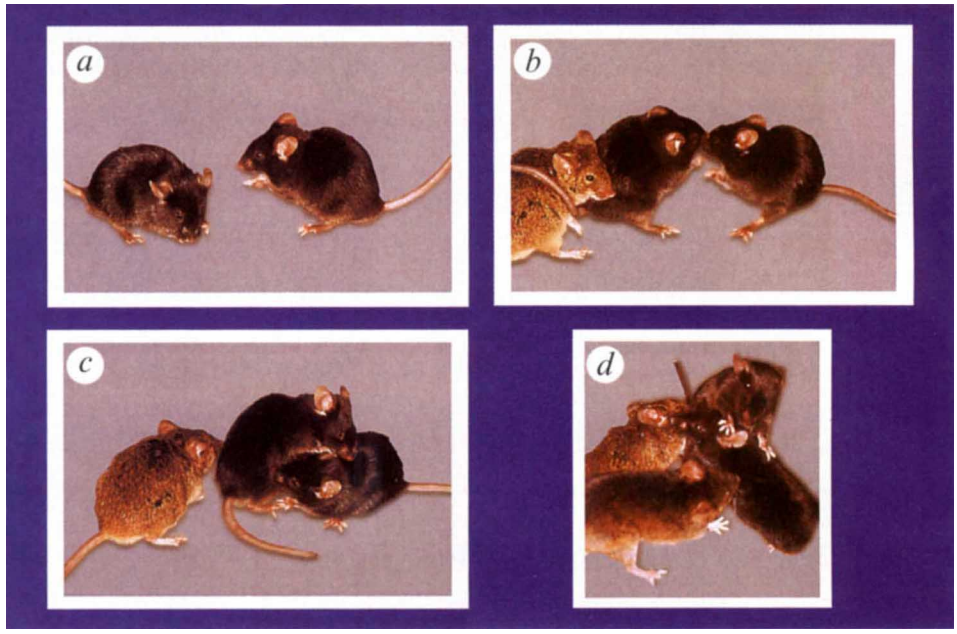

FIG. 1 Photographs of nNOS$^-$ male mice engaged in an aggressive encounter. In *a*, the mice are preparing to engage. Both black nNOS$^-$ mice in *b* are in the classic boxing stance while a brown nNOS$^-$ mouse looks on. In *c*, the two black nNOS$^-$ mice are fighting, and neither mouse has displayed a submissive posture. In *d*, the two brown nNOS$^-$ mice join in the aggressive encounter. These photographs were obtained by simultaneously introducing four nNOS$^-$ mice into a neutral arena in the experimental model used in Fig. 3. The photographed interactions commenced about 10 min after the mice were placed in the aquarium. The sequence of depicted events occurred over a 2 min period. nNOS null mice were obtained from a breeding colony established at Johns Hopkins University using animals previously produced by homologous recombination[9]. All animals were screened for homozygosity of the nNOS null mutation by Southern blot analysis. Wild-type animals consisted of both age-matched C57B6J and SvEv 129 strains to control for possible strain effects because of the nNOS null mice genetic background. Additional controls consisted of age-matched heterozygous nNOS$^{+/-}$ littermates who displayed normal behaviour when males were paired together, similar to wild-type mice. Animals were 4–6 months of age at the onset of testing (sexually mature), and were maintained individually in LD 16:8 photoperiods (light on 7:00 EST) at 20 ± 2 °C temperatures and relative humidity of 50 ± 5% throughout the study. Food (Prolab 1000; Syracuse, NY) and tap water were available ad libitum. All statistical comparisons involved one-way analyses of variance (ANOVA) except where otherwise noted. Pair-wise comparisons were accomplished with Student's *t*-tests. Mean differences were considered statistically significant if $P < 0.05$. Aggression tests were conducted as described previously[14].

have decreased to levels only one-sixth those of the initial period. At these times the number of mounts by nNOS$^-$ animals continues to be 2–3 times greater than values for wild-type animals.

Both in studies of aggression and of sexual behaviour the nNOS$^-$ animals display a marked increase in inappropriate aggressive and sexual behaviour reflected in persistent fighting and mounting behaviour despite obvious signals of surrender or disinterest, respectively, by their test partners. Generally, both aggression and sexual behaviour are enhanced by elevated testosterone levels. Accordingly, we monitored blood testosterone levels in wild-type and nNOS$^-$ males 2 weeks before any behavioural testing as well as 1 h after completion of the aggression tests. Blood plasma testosterone levels do not differ between the nNOS$^-$ and the wild-type males at either time point and there is no significant difference in the levels before and after the behavioural study.

Is it possible that sensory or motor abnormalities in the nNOS$^-$ animals account for the apparent increase in inappropriate aggressive and sexual behaviour? For instance, perhaps the nNOS$^-$ animals fail to recognize social inhibitory stimuli, such as an odour emitted by a non-oestrous female. Accordingly, we monitored olfactory behaviour by assessing the ability of mice to find a cookie hidden beneath the cage bedding. Latency to discover the hidden cookies does not differ between wild-type and nNOS$^-$ males and females. Might our behavioural observations be influenced by differences in strength and agility between wild-type and nNOS$^-$ animals? We monitored the ability of mice to turn around in a blind alley, to walk across a suspended pole, and to hang from a pole (Fig. 4 legend). nNOS$^-$ males and

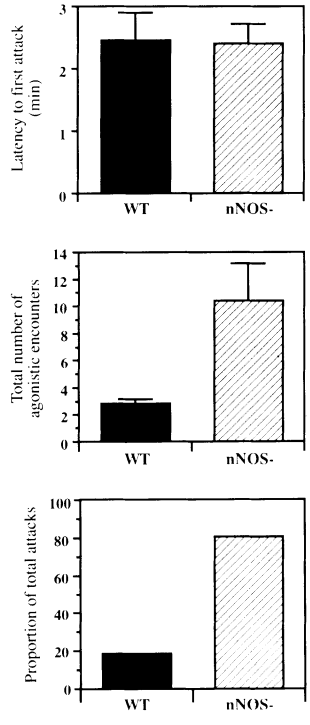

FIG. 2 Defensive aggressive behaviour in nNOS$^-$ and wild-type mice. These tests were modified from the procedures described previously to assess aggression in genetic knock-out mice[15,16]. A wild-type intruder was introduced into the home cage of either an nNOS$^-$ or wild-type adult male mouse ($n = 8$), and the defensive aggressive behaviours were recorded. The latency to first aggressive encounter, the number of agonistic encounters, and the proportion of aggressive behaviours initiated by the resident male during the 5-min tests were recorded. Aggression tests were conducted each day for three consecutive days between 15:00 and 17:00, and a unique pairing was made for each test. The data (mean ± s.e.m.) were combined from the three tests and plotted in this figure. All behavioural tests were scored blindly.

females do not display any decrease in performance in these measures. Conceivably the nNOS$^-$ animals are frightened, leading to increased fighting behaviour. We monitored behavioural anxiety in our open field test. We observe no difference between nNOS$^-$ and wild-type males and females.

Mice with targeted disruption of genes for calcium/calmodulin kinase II (ref. 15) and serotonin receptor 5-HT$_{1B}$[16] also display enhanced inter-male aggression. Augmented aggression is not likely to be a general feature of animals with targeted disruption of any gene, as animals with deletion of genes for oestrogen receptors exhibit reduced aggressive behaviour[17]. In some instances[15] mice with gene deletions display major anatomical and physiological abnormalities and appear 'sick', which can complicate interpretations of discrete behavioural deficits. For instance, mice with deleted monoamine oxidase A display increased intermale aggression but also tremble, have difficulty in righting themselves, are fearful and blind[21]. No neuroanatomical or physiological disturbances have been observed in the nNOS mice whose behaviour superficially appears normal[9].

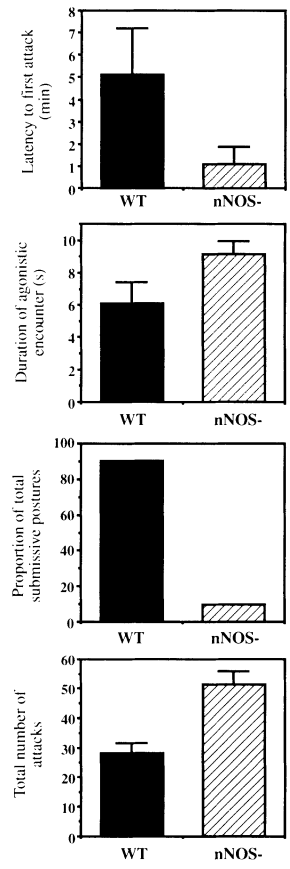

FIG. 3 Offensive aggressive behaviour among grouped nNOS$^-$ and wild-type male mice in a neutral area. Four adult male wild-type or four nNOS$^-$ ($n=16$) mice were simultaneously introduced into a clear glass aquarium (38.5 × 26.5 × 30.7 cm). The latency to first aggressive encounter, the duration of each agonistic encounter, the proportion of submissive postures, and the total number of agonistic encounters during the 15 min test were recorded for the entire group of four mice. Aggression tests were conducted on two consecutive days between 11:00 and 13:00, and the data (mean ± s.e.m.) were combined for this figure. All behavioural tests were scored blindly.

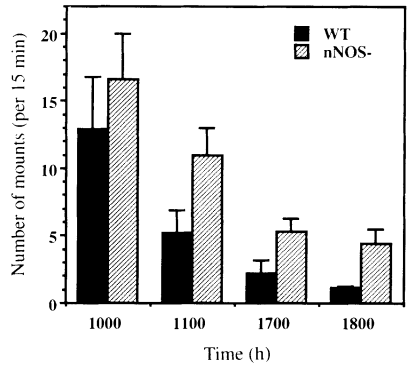

FIG. 4 Sexual behaviour of nNOS$^-$ and wild-type mice. The mean (± s.e.m.) number of mounts (per 15 min observation period) were monitored for nNOS$^-$ ($n=8$) or wild-type ($n=8$) male mice when paired with a non-oestrous female in a neutral arena. Males were initially placed in a clear aquarium (38.5 × 26.5 × 30.7 cm) for a 15 min acclimatization period. After this a non-oestrous wild-type female was introduced into the mating arena. Animals remained paired for 8 h from 10:00 to 18:00. The number of mounts and aggressive behaviours were recorded by two observers, who were uninformed about the genotype of the mice, during a 15 min observation period at the start of each hour. The behavioural tests were also videotaped continuously for subsequent confirmation of the live scoring. A mount was operationally defined as the male assuming the copulatory position, but failing to achieve intromission. Intromission was defined as the male's penis entering the vagina in association with thrusting behaviour. No animals displayed an intromission. There was only one observed incidence of aggressive behaviour, and it was performed by a nNOS$^-$ mouse. Mean (± s.e.m.) blood plasma testosterone levels of nNOS$^-$ and wild-type male mice before the start of the behavioural testing were 1.95 ± 0.22 and 2.14 ± 0.14 ng ml^{-1}, respectively ($P>0.05$); at the end of the experiment, mean (± s.e.m.) testosterone values of nNOS$^-$ and wild-type males were 1.86 ± 0.12 and 1.98 ± 0.01 ng ml^{-1}, respectively ($P>0.05$). The basal blood sample was obtained 10 days after the mice arrived in the behavioural laboratory and 2 weeks before the onset of behavioural testing. Another sample was obtained 1 h after the behavioural tests were completed. Blood samples were obtained from the retro-orbital sinus of mice that were lightly anaesthetized with methoxyflurane vapours (Metofane; Pitman Moore, Mundelein, IL). Blood was centrifuged at 3,500 r.p.m. for 1 h at 4 °C; plasma was separated and stored frozen (−80 °C) until assayed for testosterone. Blood plasma testosterone levels were assayed by radioimmunoassay (RIA) using an ^{125}I kit purchased from ICN Biochemicals (Carson, CA). The testosterone assay was highly specific; crossreactions with other steriod hormones were <0.1%. The plasma testosterone values were determined in a single RIA. Because the s.e.m. exceeded the mean, a log-transform of the data was performed and statistical analysis of these transformed data were conducted. Many tests were used to assess coordinated behaviours, including orienting reactions, forelimb strength, coordination, climbing and locomotion. All sensorimotor tests were scored blindly. In one test of agility, the time required to turn in a blind alley (up to 2 min) was recorded. The mouse was placed facing the back wall of an alley (3 cm wide with walls 15 cm high). There was no difference between nNOS$^-$ and wild-type mice in this task (22.0 ± 0.2 versus 22.3 ± 0.3 s, respectively; $P>0.05$). To assess locomotor balance and coordination, a mouse was placed at the centre of a wooden 'bridge' 60 cm long suspended 60 cm above a foam pillow. A pole bridge (2 cm in diameter) was used and the time taken for each mouse to reach the platform on either end of the bridge within 120 s was recorded. If the mouse fell, then the latency to fall within 120 s was recorded. There were no significant decreases in the performance of nNOS$^-$ mice as compared to wild-type mice. To assess forelimb strength, a mouse was suspended by its forelimbs on a wire stretched between two posts 60 cm above a foam pillow. The time (s) until the mouse fell or 90 s had passed was recorded (a score of zero was assigned to animals that fell immediately; a score of 90 was given to mice that did not fall). There were two trials to this task. nNOS$^-$ mice were not significantly impaired as compared to the performance of wild-type mice ($P>0.05$). Open field activity is a commonly used measure of anxiety levels. To assess open field activity, an animal was placed in an open area (1 m^2) for 10 min. An observer recorded the movement of the mouse during the testing period. A border 4 cm from the wall was marked off and the space beyond the border was operationally defined as the open field. The amount of time spent in the open field was compared to the time the mouse was moving along the wall of the arena. There were no significant differences between nNOS$^-$ and wild-type males (9.14 versus 8.99 min, respectively; $P>0.05$) in their open field behaviour.

Though NOS has been implicated in development of cultured PC12 cells[18], iNOS rather than nNOS was primarily involved. If nNOS deletion markedly altered neuronal development, one would anticipate substantial neuroanatomical and gross psychomotor abnormalities in nNOS$^-$ mice. However, detailed neuroanatomical studies[9] and a thorough sensorimotor evaluation have not detected abnormalities. Also, synaptic plasticity has been evaluated in the nNOS$^-$ mice revealing normal long-term potentiation in hippocampal slices[10] and normal long-term depression in cerebellar cultures[11]. Accordingly, it is highly probable that the behavioural abnormalities we have observed are direct, selective consequences of the loss of nNOS and not secondary to global physiological disruptions. Though mating and aggression are largely centrally mediated, a role for peripheral and indirect influences such as the dilated stomachs of nNOS$^-$ mice cannot be completely excluded[9]. As drug-induced serotonin depletion augments aggressive and sexual activity[22,23] and NO neurons contact serotonin cells[5], the nNOS$^-$ mice behavioural abnormalities might involve serotonin.

Our findings provide the first evidence for a behavioural role of central nNOS neurons, which presumably participate in sexual and aggressive behaviour. Studies administering NOS inhibitors have implicated NO in alcohol consumption[19] and feeding behaviour[20]. However, NOS inhibitors such as L-nitroarginine influence macrophage and endothelial NOS as well as nNOS and can affect other biological systems that use arginine. The use of mice with gene deletions overcomes such problems of nonspecificity.

Though direct comparisons are not feasible, the sexual and aggressive aberrations of nNOS$^-$ mice seem more pronounced than those reported with deletion of other genes. Accordingly, NO may be a major mediator of sexual and aggressive behaviours, relevant for studies of their biological determination in humans as well as mice. Studies of monoamine oxidase A indicate the relevance to humans of aggressive behaviour in mice. Mice with deletions of the gene for this enzyme display excessive aggression[21], and humans with low levels of the enzyme are also hyperaggressive[24]. □

Received 6 July; accepted 11 October 1995.

1. Moncada, S. & Higgs, A. *New Engl. J. Med.* **329,** 2002–2012 (1993).
2. Ignarro, L. J. *A. Rev. Pharmacol. Toxicol.* **30,** 535–560 (1990).
3. Nathan, C. *FASEB J.* **6,** 3051–3064 (1992).
4. Nathan, C. & Xie, Q.-W. *Cell* **78,** 915–918 (1994).
5. Dawson, T. M. & Snyder, S. H. *J. Neurosci.* **14,** 5147–5159 (1994).
6. Bredt, D. S. *et al. Neuron* **7,** 615–624 (1991).
7. Vincent, S. R. & Kimura, H. *Neuroscience* **4,** 755–784 (1992).
8. Rodrigo, J. *et al. Phil. Trans. R. Soc.* B**345,** 175–221 (1994).
9. Huang, P. L. *et al. Cell* **75,** 1273–1286 (1993).
10. O'Dell, T. J. *et al. Science* **265,** 542–546 (1994).
11. Linden, D. J. *et al. J. Neurosci.* **15,** 5098–5105 (1995).
12. Huang, Z. *et al. Science* **265,** 1883–1985 (1994).
13. Irikura, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **92,** 6823–6827 (1995).
14. Brain, P. F. & Haug, M. *Psychoneuroendocrinology* **17,** 537–551 (1992).
15. Chen, C. *et al. Science* **266,** 291–294 (1994).
16. Saudou, F. *et al. Science* **265,** 1875–1878 (1994).
17. Ogawa, S. *et al. Endocr. Soc. Abstr.* (1995).
18. Peunova, N. & Enikolopov, G. *Nature* **375,** 68–73 (1995).
19. Rezuani, A. H. *et al. Pharmacol. Biochem. Behav.* **50,** 265–270 (1995).
20. Squadrito, F. *et al. Eur. J. Pharmacol.* **255,** 51–55 (1994).
21. Cases, O. *et al. Science* **268,** 1763–1766 (1995).
22. Albert, D. J. & Walsh, M. L. *Neurosci. Biobehav. Rev.* **8,** 5–24 (1984).
23. Salis, P. J. & Dewsbury, D. A. *Nature* **232,** 400–440 (1971).
24. Brunner, H. G. *et al. Science* **262,** 578–580 (1993).

ACKNOWLEDGEMENTS. We thank V. B. Mountcastle for valuable suggestions. Supported by USPHS grants, a Clinical Investigator Development Award, and an International Life Sciences Foundation grant to T.M.D.; American Foundation of AIDS Research, Alzheimer's Association, National Alliance for Research on Schizophrenia and Depression grants to V.L.D.; a USPHS grant, a contract and grants of the W. M. Keck Foundation and the Stanley Foundation Research Awards program to S.H.S.; USPHS grants to R.J.N. and to Hopkins Population Center (supported testosterone RIAS) and NIH and Bristol-Myers-Squibb grants to P.L.H. and M.C.F.

Multiple essential functions of neuregulin in development

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NEUREGULIN **(also called NDF, heregulin, GGF and ARIA) is a member of the EGF family which induces growth and differentiation of epithelial, glial and muscle cells in culture[1–4]. The biological effects of the factor are mediated by tyrosine kinase receptors. Neuregulin can bind directly to erbB3 and erbB4 and receptor heterodimerization allows neuregulin-dependent activation of erbB2 (refs 1, 2, 5). A targeted mutation in mice reveals multiple essential roles of neuregulin in development. Here we show that neuregulin −/− embryos die during embryogenesis and display heart malformations. In addition, Schwann cell precursors and cranial ganglia fail to develop normally. The phenotype demonstrates that *in vivo* neuregulin acts locally and frequently in a paracrine manner. All cell types affected by the mutation express either erbB3 or erbB4, indicating that either of these tyrosine kinase receptors can be a component in recognition and transmission of essential neuregulin signals.**

To analyse the function of neuregulin *in vivo*, we have mutated the neuregulin gene by homologous recombination in embryonic stem (ES) cells, and used these cells to create mice that carry the mutation. A variety of different protein isoforms are produced from the single neuregulin gene[6]. All contain an EGF-like domain sufficient for biological activity, of which α- and β-variants exist[2]. In a first mutant allele, neuregulin$^{\Delta 7\ 9}$, the neomycin resistance (*neo*) gene replaces exon 7, 8 and 9, which encode the carboxy-terminus of the EGF domain of all known variants (Fig. 1*a*). In a second mutant allele, exon 6 is fused to β-galactosidase (neuregulin–LacZ). Exon 6 sequences downstream of the fusion point, which encode the amino terminus of the EGF-like domain of all known isoforms (Fig. 1*b*), are deleted. Healthy and fertile mice that carry either neuregulin mutation in a heterozygous state were generated. Matings between heterozygous animals produced no homozygous mutant offspring, indicating that such animals die during development. Genotyping of embryos revealed a mendelian ratio of homozygous mutant embryos up to and including day 10.5 in embryogenesis (E10.5). The survival of homozygous mutant embryos declined sharply, and none were alive on E11.5. They were found dead *in utero*, as judged by signs of resorption and absence of heartbeat. Analysis of mutant neuregulin locus (Fig. 1*c*) and mutant transcripts (Fig. 1*d*) demonstrate the presence of the desired mutation in the animals. Homozygous neuregulin–LacZ and neuregulin$^{\Delta 7\ 9}$ embryos display identical phenotypic changes.

Viable neuregulin −/− embryos on E10.5 were normal in overall size, but had irregular heartbeat and, frequently, an enlarged heart or pericard. Histological analysis showed that they had poorly developed ventricular trabecules (Fig. 2*a*, *b*) but unchanged morphology of the atrium. Trabecules are the first morphological sign of ventricle differentiation and are formed by heart muscle cells[7]. Because of the missing trabecules, the ventricle and atrium had a similar morphology in neuregulin −/− embryos on E10.5, the endocardial cushion was not closed, and mesenchymal cells forming the cushion were reduced in number (Fig. 2*a*, *b*).

Expression analysis of neuregulin, erbB3 and erbB4 demonstrated the presence of all three molecules in the developing heart. X-Gal staining of neuregulin–LacZ/+ embryos, or *in situ* hybridization analysis with neuregulin-specific probes, demonstrated that neuregulin is produced in the endocardial endothelium (Fig. 2*c*, *f*), which does not express erbB3 or erbB4. Particularly strong neuregulin expression is associated with

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