Resolving differences in $GABA_A$ receptor mutant mouse studies

TO THE EDITOR—The validity of genetically altered mice as models for disease or for drug target identification relies on the reproducibility of behavioral test results. In a recent paper in *Nature Neuroscience*[1], behavioral tests were described on α1(H101R) mice carrying a histidine-to-arginine point mutation in the α1 subunit of the $GABA_A$ receptor. An accompanying News and Views article[2] discussed discrepancies between results of behavioral experiments for this study[1] and our study of α1(H101R) mice[3]. The two lines of mice seemed to differ in their drug-induced behavior, although both had been constructed with the same point mutation. Now we report that these discrepencies were caused by differences in the behavioral protocols used by the two groups, not by differences in the mouse lines.

Both studies compared the behavioral effects of diazepam in wild-type and point-mutated α1(H101R) mice whose α1 $GABA_A$ receptor subtype was made insensitive to diazepam by a histidine-to-arginine substitution. We assessed the sedative action of diazepam on spontaneous motor activity of mice familiar with the testing environment[3]. Diazepam produced sedation in wild-type mice, as measured by the dose-dependent decrease in the extent of spontaneous motor activity, whereas diazepam failed to impair the spontaneous motor activity in α1(H101R) mice up to 30 mg per kg (ref. 3). These results led us to conclude that the sedative action of diazepam as measured by this protocol is mediated by α1 $GABA_A$ receptors. In contrast, McKernan and colleagues measured locomotor activity in mice exploring an unfamiliar environment[1]. The authors reported that diazepam (3 mg per kg orally) did not affect wild-type mice, but induced a significant increase in locomotor activity in the α1(H101R) mice. This has been interpreted as "paradoxical hyperactivity"[2] and "reduction in neophobia"[1] or "reduction in GABA-mediated neuronal inhibition"[1]. Following transfer to a new testing room 30 minutes before drug treatment, our α1(H101R) mice also exhibit hyperactivity (**Fig. 1**). These findings, which correspond to McKernan and colleagues' results[1], suggest that the failure of diazepam to interact with α1 receptors is associated with a heightened behavioral arousal in response to stressors in α1(H101R) mice.

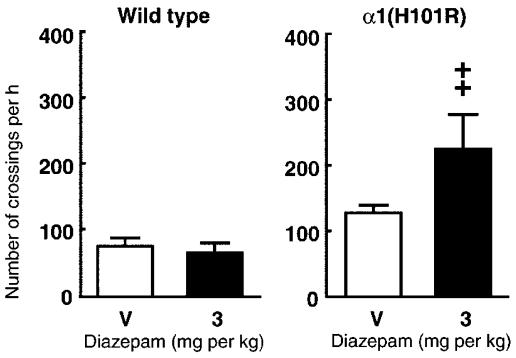

Fig. 1. Enhanced locomotor activity of α1(H101R) mice compared to wild-type mice in a novel testing environment 30 minutes following diazepam treatment (3 mg per kg orally). Locomotion was recorded in an automated two-chamber apparatus for one hour. Results are expressed by mean number of crossings ± s.e.m. (n = 6–9 per group; genotype × treatment, $F_{1,26}$ = 4.94, ++ p < 0.01, Newman-Keuls test).

Another discrepancy between the studies was the reduced sensitivity of McKernan and colleagues' mutant mice[1] to the ataxic effect of diazepam in locomotor performance during a rotarod test, in which the rod rotated at 18 revolutions per minute (rpm). In our test, at 2 rpm, motor coordination remained unaltered in the mutant as compared to wild-type mice[3]. However, when we conducted the rotarod test at a higher speed (4–40 rpm), the α1(H101R) mice remained insensitive to diazepam up to 10 mg per kg (**Fig. 2**). These findings are similar to those reported by McKernan and colleagues[1].

Thus, under comparable test conditions, there are no apparent behavioral differences between the α1(H101R) mice generated by the two laboratories[1,3]. This shows that taking environmental and technical details of test procedures into account may help to resolve interlaboratory differences in results.

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3. Rudolph, U. *et al. Nature* **401**, 796–800 (1999).

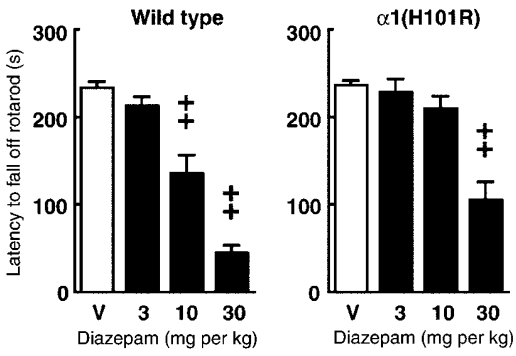

Fig. 2. Motor-impairing effect of diazepam in a 4–40 rpm rotarod test (10 steps of 25 seconds each) in wild-type and α1(H101R) mice. Mice pretrained to walk on the rotating rod (up to 28 rpm) were treated with either vehicle or diazepam (3, 10 or 30 mg per kg) and tested on the rotarod 30 minutes later. Results are expressed by the mean latency to fall off the rotarod ± s.e.m. (n = 16 per group; genotype × treatment, $F_{3,120}$ = 3.06, ++ p < 0.01, Newman–Keuls test).