Between-subject transfer of emotional information evokes specific pattern of amygdala activation

Ewelina Knapska*, Evgeni Nikolaev†, Pawel Boguszewski*, Grazyna Walasek*, Janusz Blaszczyk*, Leszek Kaczmarek†‡, and Tomasz Werka*

Departments of *Neurophysiology and †Molecular and Cellular Neurobiology, Nencki Institute, Pasteur 3, PL-02-093, Warsaw, Poland

Communicated by Jan Bureš, Czech Academy of Sciences, Prague, Czech Republic, January 2, 2006 (received for review October 20, 2005)

Emotional states displayed by an animal or a human can seriously affect behavior of their conspecifics. The amygdala plays a crucial role in the processing of emotions. In this study, we describe an experimental rat model of between-subject transfer of emotional information and its effects on activation of the amygdala. The rats were kept in pairs, and one animal (designated as ''demonstrator'') was treated to specific behavioral training of either foot-shock-reinforced context conditioning or just exposure to a novel context. We next examined the influence of the demonstrators on the exploratory behavior of their cagemates (called ''observers'') and the observers' performance of the acoustic startle response. We report that we can distinguish both groups of observers from the control animals (as shown by startle-response measure) and distinguish between observers (by means of indexing the exploration), with respect to whether they were paired with demonstrators treated to different experimental conditions. Furthermore, we show that the observers have most of their amygdala activated (as revealed by c-Fos mapping) to the same level as the demonstrators and, in the case of the central amygdala, to an even higher level. Moreover, the level of c-Fos expression in the observers reflected the specific behavioral treatment of the demonstrators with whom they were paired. Thus, in this study, we have shown that undefined emotional information transferred by a cohabitant rat can be evaluated and measured and that it evokes very strong and information-specific activation of the amygdala.

c-Fos | emotion | social communication | brain mapping | empathy

Emotions coordinate homeostasis of an organism in a complex, dynamic environment and participate in regulation of social behaviors. Emotional states displayed by an animal or a human can seriously affect the behavior of conspecifics. This fact has been demonstrated in numerous studies involving simulated and real panic situations, in which the presence of a leader determines the time of achieving the goal of a safe exit (1, 2). The escape panic could happen in life-threatening situations, such as fires in crowded buildings, but sometimes, interestingly, it seems to emerge without any apparent cause. This kind of panic is probably provoked by the specific emotional behavior of some members of the crowd.

It is well known that the elaborate emotional systems of social species, such as humans, allow the recognition of very subtle emotional signals. Most of the functional imaging studies in humans have used emotional facial expressions as social signals presented to a subject to associate differences in the social content of stimuli with differences in the activity of the neural structures engaged in the processing of such stimuli (3). The results of these studies clearly pointed to the amygdala being involved in the processing of negatively valenced stimuli of biological importance (4–6). The neuroimaging studies also revealed that fearful faces are especially effective in activating the amygdala. These findings may be explained by the inherent ambiguity of the fearful faces as compared to, e.g., angry faces. Angry faces provide information about the presence of a threat, but, at the same time, they also give information about the source of the threat, whereas the fearful faces provide less precise information, only about the presence of the threat (5).

Many studies on animals, performed mostly in the contextual fear-conditioning paradigm, have shown that the amygdala plays a crucial role in aversive conditioning, because it is involved in the processing of emotions and associating perceptual representations of stimuli with emotional response, cognitive processing, and behavioral motivation (7). However, the involvement of this structure in perceiving and processing emotional stimulation of less well defined consequences, such as information transferred by the conspecifics behaving fearfully, have not been studied systematically. Interestingly, the experiments of Cook and Mineka (8) demonstrated that observation of model monkeys exhibiting an intense fear of fear-relevant stimuli not only changed the present behavior of an observer rhesus monkey but also resulted in long-lasting fear to observed stimuli. Moreover, the findings of animal and human studies provide evidence that the amygdala modulates the consolidation of long-term memories of emotionally arousing experiences (9).

Motivated by the observations described above, we wanted to establish an experimental animal model of between-subject transfer of emotional information. In the present study, we examined whether emotional information transmitted by one rat, previously trained in the contextual fear-conditioning paradigm, can influence the behavior of the observers. We used two behavioral measures: (*i*) performance of acoustic startle response, which has been shown to be enhanced in animals by fearful stimuli (10) and (*ii*) intensity of exploration. Then, we checked whether the emotional information evokes activation of the amygdala of the other animal. We used a c-Fos immunolabeling method, which provides a mapping tool, enabling a single-cell resolution, thereby allowing determination of the involvement of separate brain regions, and even their subdivisions, in specific behavioral responses.

Results

Behavioral Results. In all of the experiments described herein, we have examined how information about current experience is passed on from one animal to another. One of the pair of rats (''demonstrator'') was exposed to the specific behavioral condition [either to context fear conditioning, in which the animal received a foot-shock (S-d), or to control exposure to a new cage, nonshock (NS-d)] (Fig. 1). Then the demonstrator returned to the home cage where it was in contact with the cagemate (designated as ''observer,'' either S-o or NS-o). The reaction to the demonstrator was analyzed by either measuring the observer's activity to approach and explore the demonstrator or by testing the acoustic startle response of the observer (11); both behaviors were investigated just after reunification with the

Conflict of interest statement: No conflicts declared.

Abbreviations: ASR, acoustic startle response; H, habituated; NS, nonshocked; NS-d, NS demonstrator; NS-o, NS observer; S, shocked; S-d, S demonstrator; S-o, S observer.

‡To whom correspondence should be addressed. E-mail: l.kaczmarek@nencki.gov.pl.

www.pnas.org/cgi/doi/10.1073/pnas.0511302103

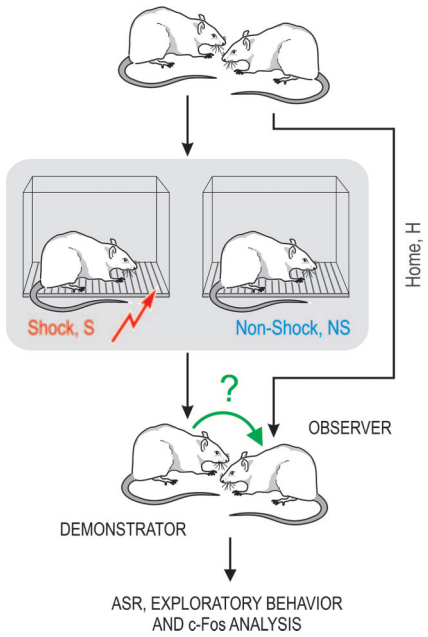

Fig. 1. Schematic representation of the experimental procedures used.

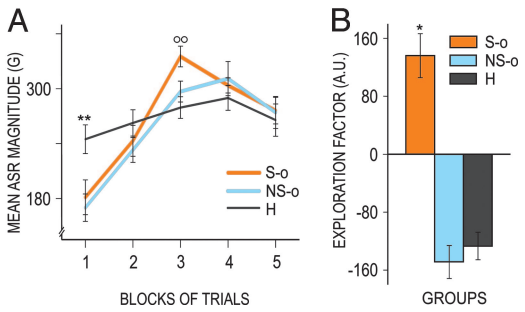

Fig. 2. Behavioral measures allowing investigation of the between-subject transfer of emotional information. (*A*) Mean ASR amplitudes ± SEM in the observer rats that were paired with demonstrator rats either receiving foot-shock (S-o), or not (NS-o) and H groups in five consecutive blocks of trials (four trials each). The three-way ANOVA revealed the following interactions: (*i*) subgroup × block of trials ($F_{(4,168)} = 6.94$, $P < 0.0001$) and (*ii*) the group × subgroup × block of trials ($F_{(8,168)} = 2.28$, $P < 0.02$). The effect of block of trials ($F_{(4,168)} = 17.36$, $P < 0.0001$) was also seen. The differences in temporal characteristics of the startle-response magnitude between the S-o, NS-o, and H groups were confirmed by two-way ANOVA, which revealed group × block of trials interaction ($F_{(8,84)} = 2.79$, $P < 0.01$). Further Duncan post hoc tests showed differences in ASR amplitudes between the N and NS groups in comparison with the H group in the first block (**, $P < 0.01$) and between the S and H groups in the third block (○○, $P < 0.01$) of trials. Similar two-way ANOVA for demonstrators yielded neither group and block effects nor interaction. (*B*) The magnitude of exploration factor in the observers from the S-o, NS-o, and H groups. The exploration factor was calculated as a difference between the head movements made in the first 2 min after reunion of the demonstrators and the observers in the testing session and the head movements during the first 2 min of the preceding session. The three-way ANOVA was used to compare mean distances of head movements in the observers from the S-o, NS-o, and H groups in pretesting and testing sessions and two 2-min time intervals. Clear differences between groups were seen. Either group × session ($F_{(2,21)} = 4.52$, $P < 0.02$) or group × time intervals interactions ($F_{(2,21)} = 4.13$, $P < 0.03$) and the effect of time intervals ($F_{(1,21)} = 43.47$, $P < 0.0001$) were yielded. Bars denote ± SEM. *, $P < 0.05$.

demonstrator that was treated as described above (Fig. 1). In addition, control animals that were habituated (H) to a brief separation were also investigated. In the H group, no difference between demonstrators and observers could be noted, as far as the behavioral and immunocytochemical tests described below are concerned.

The first behavioral measure, the acoustic startle response (ASR), showed no difference between the observers of information that were exposed to either the S-d or NS-d partners (Fig. 2*A*). The dynamics of these responses were, however, clearly different from that recorded in H rats. At the beginning of ASR testing (block 1), S-o and NS-o animals responded with significantly lower amplitudes of the startle in comparison with H subjects. However, this initially low level was followed by gradual increase in the subsequent blocks of trials that eventually, in the case of the S-o group and block 3, exceeded that for control H rats (Fig. 2*A*).

In contrast, the second behavioral measure, exploration intensity, differentiated the observers from the S-o group from the other two groups. In the observers from the S-o group, there was a clear enhancement of exploration (Fig. 2*B*), whereas a systematic decrease of this reaction was noted in the NS-o and H animals. Qualitatively, the observers from the S-o followed and explored the demonstrators, and the exploration intensity reflected the increase of movement of the whole pair of animals, whereas, in the NS and H groups, they moved rather independently. Hence, the exploration intensity appears to serve as an appropriate measure to account for the animals' arousal.

c-Fos Expression. In all amygdalar nuclei, c-Fos expression was clearly enhanced in the S and NS groups (in both the demonstrators and the observers) in comparison with the H group. The level of c-Fos expression was higher in the S group (both S-d and S-o) than in the NS group (NS-d and NS-o) in all but the central and cortical nuclei, where the level of expression was similar in both groups. Most notably, there were no differences in c-Fos expression between the observers and the demonstrators, except for the central nucleus, where, surprisingly, higher levels were shown in the observers. The representative amygdala sections are shown in Fig. 3*A*, whereas the results of quantitative and statistical analysis of c-Fos expression are presented in Fig. 3*B*.

Discussion

In this study, we have described an experimental rat model of between-subject transfer of emotional information. The rats were kept in pairs, and one animal was treated to specific behavioral training of either foot-shock reinforced context conditioning or just exposure to a novel context. Next, the animals were reunited. Then, we examined the influence of the demonstrators on exploratory behavior of the observers and the observers' performance of the ASR. We report that there is, indeed, a transfer of emotional state between subjects, and we can distinguish (*i*) the observers of information from control animals (as shown by startle response measure) and (*ii*) the observers from the S and NS groups (by means of indexing the exploration), with respect to whether they were paired with demonstrators treated to different experimental conditions. Furthermore, we show that the observers have most of their amygdala activated (as revealed by c-Fos mapping) to the same level as the demonstrators, reflecting their specific behavioral treatment and, in the case of the central amygdala, to even higher levels.

We documented a different pattern of ASR in the observers from both the shocked and NS animals as compared with the H animals. Startle, a fast motor response to sudden, intense, unexpected stimuli is a sensitive measure of the emotional state of the organism, correlated with the fear-related amygdalar activity (10). The clear within-testing-session sensitization of the startle response in the observers in the present experiment,

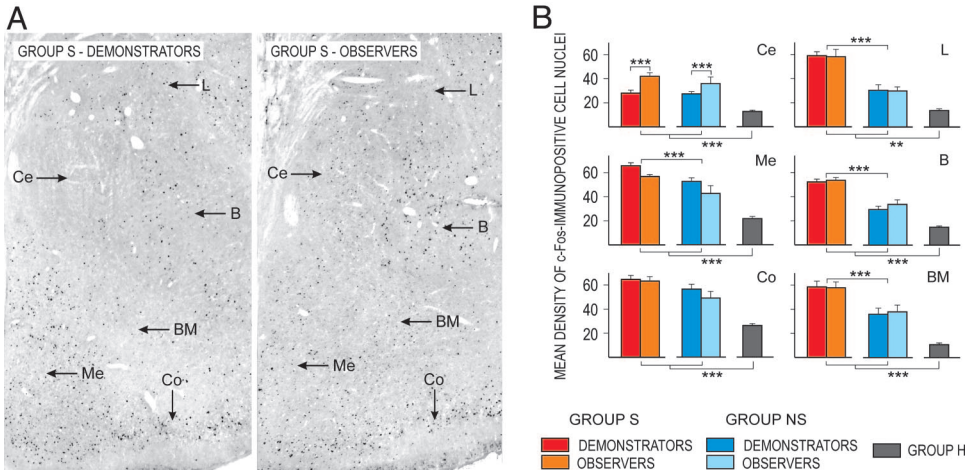

Fig. 3. c-Fos expression in the amygdala. (*A*) c-Fos immunoreactivity (black dots) observed in the amygdala of demonstrators and observers from the S group. (*B*) Mean number of c-Fos-immunopositive cell nuclei ± SEM; Ce, central nucleus; Me, medial nucleus; Co, cortical nuclei; L, lateral nucleus; B, basal nucleus; BM, basomedial nucleus. Three-way ANOVAs that were independently performed for each amygdalar nucleus revealed group effects ($P < 0.0001$). The subgroup effect was yielded only in the Ce nucleus ($P < 0.0001$). Neither the brain-slices effect nor double or triple interactions were seen. The levels of significance (Duncan tests) between groups and subgroups are shown. **, $P < 0.01$; ***, $P < 0.001$.

proved the strong impact of such undefined information on the emotional state and perception of stimuli by the animals. It is also interesting that a similar level of enhancement was seen in the observer rats, irrespective of whether they were paired with either S or NS cagemates. It should be noted, however, that the demonstrators from the NS group experienced a novel, stressful situation. On the other hand, we also noted changes in the observers' behavior that were specific for rats from the S group. Namely, in the first minutes after the reunion, the observers of information from this group were exploring the cagemates more intensely than the observers from the NS and H groups, which explored even less than in the previous session.

Furthermore, we studied the involvement of amygdalar nuclei in the processing of transmitted emotional information by measuring c-Fos expression. The level of c-Fos expression in the observers was surprisingly high in all amygdalar nuclei. Most interestingly, in the medial, lateral, basal, and basomedial nuclei of the amygdala, the c-Fos expression in the observers reflected the demonstrators' treatment, i.e., it was higher in the observers receiving information from the demonstrators obtaining footshocks (S-o) compared with the NS group (NSo). Surprisingly, the demonstrators did not express more c-Fos than the observers; on the contrary, in the central nucleus, the observers expressed more c-Fos than the demonstrators in both the S and NS groups. These results are consistent with the results of neuroimaging studies, in which fearful faces were especially effective in activating the amygdala (5). The gradual enhancement of the acoustic startle seen in the observers in our experiment also indicates that transmitted emotional information strongly affects its observers.

It is also interesting to compare the pattern of activation of the amygdalar nuclei evoked by strongly aversive vs. less-aversive stimulation. We observed that the basolateral part of the amygdala (the lateral, basal, and basomedial nuclei) and the medial nucleus responded more strongly to the purely aversive stimulation than to the much less-aversive stimulation in the NS group. In contrast, the central and cortical nuclei were activated at similar levels by both kinds of stimulation. These results seem to be consistent with the notion that the basolateral complex of the amygdala is particularly important for creating current stimulus-value associations, which support instrumental behavior, helping the animal to adapt to the changing environment (12). As proposed by Bechara *et al.* (13), who studied the involvement of the human amygdala in the gambling task, the essential contribution of this structure consists in evoking appropriate emotional states, which guide response selection. Our result suggests that it is the basolateral complex of the amygdala that is particularly sensitive to the changes in the current value of not only well defined fearful stimuli but also of less well defined anxiety-like stimuli, as suggested in ref. 14. Information transferred from the demonstrators to the observers in the NS group was probably also stressful to some extent (see the startle-response magnitude in the observers) but, obviously, to a lesser degree than in the S group. Taking this into account, the level of c-Fos activation reflected precisely the current value of stimuli in both the demonstrators and the observers. The strong activation of the basolateral complex of the amygdala in either the demonstrators or the observers is also consistent with the role of this structure in modulating the consolidation of memory of emotionally arousing experiences (9).

In the central nucleus of the amygdala, the level of c-Fos expression in the observers of information was clearly higher than in the demonstrators. Thus, the central amygdala seems to be specifically activated in the processing of unspecified information and, to a lesser degree, by novelty, but not by fear learning. This finding is in apparent contrast to the results of many earlier studies that reported the role of the central nucleus in classical fear conditioning (15). Generally, the central nucleus of the amygdala, which has extensive projections to numerous nuclei in the midbrain and brainstem, has been seen for many years as the key structure to orchestrate behavioral, autonomic, and endocrine responses to threat and danger (7). However, the lack of involvement of this nucleus in learning of the aversively motivated behaviors has also been observed (14, 16, 17). On the other hand, as measured by c-Fos expression, the central nucleus is involved in retrieval of contextual conditioned fear (18). These discrepancies may result from the different methods used in these studies (lesion vs. c-Fos expression, different conditioning paradigms) but also from damage of the fibers of passage in the central nucleus in the lesion studies (see ref. 19 and discussion therein). Hence, it is important to note that many effects attributed to the central nucleus may actually result from disconnecting the basolateral nucleus from the bed nucleus of the stria terminalis, because the fibers that connect these structures pass right through the central nucleus (5). It is also worth noting that the central nucleus has been shown to play a role in

attentional processes (20, 21), which are particularly important in situations of uncertain but potentially significant consequences, as in the observers from our study.

The emotional information perceived by the observers had much less well defined consequences in comparison with stimuli affecting the demonstrators during fear conditioning. Thus, it seems that the experimental situation evoked an emotional state more akin to anxiety than to fear in observers. This statement is consistent with the results of Sullivan *et al.* (22), who showed activation of the central nucleus of the amygdala, measured by c-Fos expression, in rats after the anxiogenic drug doxapram.

Because c-Fos is a product of an immediate early gene and a component of a transcription factor (AP-1), it may orchestrate expression of a number of other genes, and, thus, c-Fos can also be treated as a marker of neuronal plasticity (for details see ref. 23). From the perspective of the results presented herein, it seems that the plasticity during fear conditioning results from a change in synaptic inputs in the basolateral amygdala rather than from the changes in its efferent target areas like the central nucleus. This point of view is further supported by our previous results on c-Fos expression in different behavioral models as well as by the results obtained with the electrical stimulation of the amygdala (23–26).

Incidentally, our results call for reappraisal of number of the previous studies, in which c-Fos was investigated in a context of conditioning (23). In the case of experiments involving animals kept in groups and taken for behavioral training individually and then returned to the cagemates, it appears that their c-Fos expression patterns should be reconsidered, especially as far as the central amygdala is concerned (see ref. 23 for the extensive list of appropriate literature).

The nature of transferred information is a question that has not been addressed in this study. However, we may suppose that information could be transferred by alarm pheromones. Recently, Kiyokawa *et al.* (27) showed that alarm-pheromones perception induces c-Fos expression in the medial, lateral, and basal nuclei of the amygdala. Interestingly, the level of c-Fos expression in these nuclei in our experiment happened to mirror the demonstrator's treatment. It also seems consistent with our result that the observers from the S-o group explored their partners more than the observers from the NS-o group.

Another possible way to transmit information is ultravocalization. It is well documented that rats emit ultrasonic calls and that the frequency and duration of such vocalizations are determined by specific environmental stimuli. These calls can, therefore, have an important communicative role (28, 29). Indeed, we recorded 22-kHz ultrasonic vocalization during the first minutes after the trained rat was placed back into the home cage (data not shown). It is also known that the presentation of such ultrasounds can induce defensive behavior and c-Fos expression in the amygdala (30). Obviously, information could also be transferred in other ways, such as tactile stimulation or distinctive patterns of behavior. This issue requires additional studies.

In conclusion, in this study, we have shown that undefined information transferred by a cohabitant rat can evoke very strong activation of the amygdala. The pattern of the activation in the amygdalar nuclei appears to be different with regard to the kind of information, with the central nucleus more involved during less well defined stimulation and the basolateral and medial nuclei activated by both defined and undefined information. The ASR was enhanced in observers of information from both S and NS animals. We also observed a specific pattern of behavior after returning of demonstrators to the home cages, with the observers from the S group exploring more intensely than the observers from other groups.

Materials and Methods

Subjects. The experiment was performed on 72 adult, experimentally naïve male Wistar rats (250–300 g at the beginning of the experiment), supplied by the Nencki Institute Animal House. For 1 month before the experiment, the animals were housed in pairs in standard home cages (43.0 × 25.0 × 18.5 cm) under a natural light–dark cycle, with food and water provided ad libitum. The rats were habituated to the experimenter's hand for 14 days preceding the experiment. The experiment was carried out in accordance with the Polish Act on Animal Welfare, after obtaining specific permission from the First Warsaw Ethical Committee on Animal Research. All efforts were made to minimize the number of animals and their suffering.

Procedure and Group Treatment. The animals were randomly divided into three groups (16 animals per group in the behavioral experiments and 8 animals per group for the c-Fos-expression analysis). In the behavioral experiments, all animals were habituated for 3 days (one session per day) to the experimental room and marking process. In the habituation sessions, each pair of rats was brought to the experimental room for 20 min. Then the cohabitants were separated for 10 min (each subject was replaced in the new home cage). Each animal was marked with two different-color spots (one on the head, 1 cm in diameter and one on the central part of the back, 2–3 cm in diameter), and then the pair of rats was reunited and left in the home cage in this room for 10 min. On the experimental day, in each cage, one subject was treated as a demonstrator and the other as an observer (Fig. 1). In the S group, the demonstrators (S-d) were taken from their home cages for Pavlovian contextual fear conditioning. The training was performed in an experimental cage (62.0 × 18.0 × 29.0 cm) that was housed in a sound-attenuating room. Nine foot-shocks lasting 1 s were applied with interstimulus intervals of 55 s. Current pulses of 50 Hz and 1.3-mA intensity were delivered via the grid floor (0.4-cm-diameter bars at 1.5-cm intervals) from a shock generator (Grason–Stadler model E 1064). When the demonstrators were trained, their cohabitants (observers, S-o) were kept in the home cages in a different sound-attenuating room and were not able to hear the vocalization of demonstrators. Immediately after the training, the demonstrators were placed back in their home cages and allowed to interact with the observers. The NS group, composed of rats treated as those from the S group, except that the NS demonstrators (NS-d) received no foot-shocks in the experimental cage. The animals from the third group, habituated controls (H), were habituated to the experimental room, marking process, and separation. Except for the habituation, they were in their home cages all of the time.

To briefly summarize our experimental setup, we used six subgroups: S-d, S-o, NS-d, NS-o, H-d, and H-o. In the H group, no difference between demonstrators and observers was observed; therefore, for graphical constructions, the H-d and H-o subgroups were treated as one habituated control group H.

Behavioral Experiments. The behavior of the observers from S, NS, and H groups was recorded for 10 min after the return of the demonstrator to the home cage. As a behavioral measure, we chose the total distance traveled by the head of the observer, which was justified by the qualitative features of the animals' behavior (see *Results*). The total distance traveled by the head of the observers from the S, NS, and H groups was recorded with a high-resolution color camera and MPEG-encoder PC card and stored in MPEG2 digital format. For color-dots tracking and automated behavior analysis, an image-recognition system was used (EthoVision Color Pro 3.1; Noldus, Wageningen, The Netherlands). Tracks of the head color dots were digitized, and the total distance traveled by the head was calculated (with

segmentation on time intervals). After 10 min, both the demonstrator and the observer from a given pair were tested simultaneously in an acoustic chamber.

The ASR testing was performed in a Coulbourn apparatus (Coulbourn Instruments, Allentown, PA) equipped with two force-sensitive platforms placed in a soundproof ventilated chamber. A loudspeaker located 10 cm above the cages delivered 110 dB (SPL) 20-ms white-noise pulses with 2-ms rise time, which served as the startle stimulus. Each pair of rats was tested simultaneously in two independent small plastic cages (180 × 85 × 90 mm each) placed on the force-sensitive platforms. The ground-reaction forces exerted on the platform by the animal's startle were measured. The amplified, rectified, and filtered (40-Hz low-pass filtered) signals were sampled at 400 Hz for a 200-ms poststimulus period.

After a 2-min adaptation period, the rats were exposed to a sequence of 20 acoustic stimuli arranged in a pseudorandom sequence and spaced by intertrial intervals of pseudorandom duration from 9 to 52 s. The acoustic stimuli were presented against a 70-dB white-noise background (11).

Immunocytochemical Assessment of c-Fos Expression. For the c-Fos expression analysis, the rats were killed at 90 min after the return of a demonstrator to a home cage (the S and NS groups) or directly from their home cages (the H group). These rats received no marks-tracking or other behavioral treatment before the experiment. Rats were killed by an overdose of chloral hydrate. Then, the animals were perfused intracardially with ice-cold saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and stored in the same fixative for 24 h at 4°C and subsequently immersed in 30% sucrose with 0.01% sodium azide at 4°C. Then the brains were frozen on dry ice and sectioned at 40 μm on a cryostat. The coronal brain sections containing amygdaloid nuclei, 1.0–3.3 mm posterior to bregma, were collected (31). The immunocytochemical staining was performed on free-floating sections (14). The sections were washed three times in PBS (pH 7.4; Sigma) with 0.3% Triton X-100 (Sigma), incubated for 10 min in 0.003% H_2O_2 in PBS, washed twice in PBS/Triton X-100 and incubated with a polyclonal antibody (anti-c-Fos, 1:1,000; sc-52; Santa Cruz Biotechnology) in PBS/Triton X-100 and normal goat serum (3%; Vector Laboratories) for 48 h at 4°C. The sections were then washed three times in PBS/Triton X-100, incubated with goat anti-rabbit biotinylated secondary antibody (1:1,000; Vector Laboratories) in PBS/Triton X-100 and normal goat serum (3%) for 2 h, washed three times in PBS/Triton X-100, incubated with avidin–biotin complex (1:1,000 in PBS/Triton X-100; Vector Laboratories ABC kit) for 1 h, and washed three times in PBS. The immunostaining reaction was developed by using the oxidase–diaminobenzidine–nickel method. The sections were incubated in distilled water with diaminobenzidine (DAB; Sigma), 0.5 M nickel chloride, and peroxidase (Sigma) for 5 min. The staining reaction was stopped by three washes with PBS. The reaction resulted in a dark brown staining within the nuclei of c-Fos immunoreactive neurons. The sections were mounted on slides, air dried, dehydrated in ethanol solutions and xylene, and coverslipped with Entellan (Merck).

The measure of c-Fos immunopositivity was expressed as density, determined in the following manner. For each brain section, the number of c-Fos immunopositive nuclei in a given amygdalar structure was counted and divided by the area occupied by this structure (in arbitrary units). The borders of the subnuclei were determined with the use of the Nissl-stained adjacent section. The image analysis was done with the aid of an image analysis computer program (IMAGE J) for two sections for each animal.

Data Analysis. Between- and within-group changes in the startle amplitude were analyzed by using a three-way mixed-design ANOVA for one repeated factor (five blocks, four trials each) and two independent factors (three groups and two subgroups). Additionally, two-way mixed-design ANOVAs for repeated (five blocks, four trials each) and independent (two subgroups) factors were performed independently for each group. Further post hoc Duncan tests were conducted for more detailed comparisons of observers' results. The changes between total distances moved by the head of the observer were analyzed with the use of a three-way mixed-design ANOVA for two repeated factors (pretesting and testing sessions and two 2-min time intervals) and one independent factor (three groups). The exploration factor was calculated as a difference between the distance moved by the head in the first 2 min after reunion of the demonstrators and the observers in the testing session and the distance moved by the head during the first 2 min of the preceding session.

The number of c-Fos immunopositive cell nuclei was analyzed by a three-way mixed-design ANOVA for two independent factors (three groups and two subgroups) and one repeated measure (two brain sections), independently for each amygdalar nucleus.

Further post hoc Duncan tests were performed for more detailed comparisons.

We thank Robert K. Filipkowski for helpful comments. This work was supported by State Committee for Scientific Research (KBN, Poland) Grant 6P05A01420 (to T.W.).

1. Klein, A. L. (1976) *J. Pers. Soc. Psychol.* **34,** 1143–1154.
2. Saloma, C., Perez, G. J., Tapang, G., Lim, M. & Palmes-Saloma, C. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 11947–11952.
3. Adolphs, R. (2003) *Nat. Rev. Neurosci.* **4,** 165–178.
4. Adolphs, R., Tranel, D., Damasio, H. & Damasio, A. (1994) *Nature* **372,** 669–672.
5. Davis, M. & Whalen, P. J. (2001) *Mol. Psychiatry* **6,** 13–34.
6. Zald, D. H. (2003) *Brain Res. Brain Res. Rev.* **41,** 88–123.
7. Davis, M. (2000) in *The Amygdala, A Functional Analysis*, ed. Aggleton, J. (Oxford Univ. Press, Oxford), pp. 213–287.
8. Cook, M. & Mineka, S. (1989) *J. Abnorm. Psychol.* **98,** 448–459.
9. McGaugh, J. L. (2004) *Annu. Rev. Neurosci.* **27,** 1–28.
10. Koch, M. (1999) *Prog. Neurobiol.* **59,** 107–128.
11. Blaszczyk, J. & Tajchert, K. (1996) *Acta Neurobiol. Exp. (Warsaw)* **56,** 919–925.
12. Baxter, M. G. & Murray, E. A. (2002) *Nat. Rev. Neurosci.* **3,** 563–573.
13. Bechara, A., Damasio, H., Damasio, A. R. & Lee, G. P. (1999) *J. Neurosci.* **19,** 5473–5481.
14. Savonenko, A., Filipkowski, R. K., Werka, T., Zielinski, K. & Kaczmarek, L. (1999) *Neuroscience* **94,** 723–733.
15. Fendt, M. & Fanselow, M. S. (1999) *Neurosci. Biobehav. Rev.* **23,** 743–760.
16. Duncan, G. E., Knapp, D. J. & Breese, G. R. (1996) *Brain Res.* **713,** 79–91.
17. Knapska, E., Walasek, G., Nikolaev, E., Neuhäusser-Wespy, F., Lipp, H.-P., Kaczmarek, L. & Werka, T. *Learn. Mem.*, in press.
18. Scicli, A. P., Petrovich, G. D., Swanson, L. W. & Thompson, R. F. (2004) *Behav. Neurosci.* **118,** 5–14.
19. Koo, J. W., Han, J. S. & Kim, J. J. (2004) *J. Neurosci.* **24,** 7654–7662.
20. Gallagher, M., Graham, P. W. & Holland, P. C. (1990) *J. Neurosci.* **10,** 1906–1911.
21. Holland, P. C. & Gallagher, M. (1993) *Behav. Neurosci.* **107,** 246–253.
22. Sullivan, G. M., Apergis, J., Gorman, J. M. & LeDoux, J. E. (2003) *Biol. Psychiatry* **53,** 863–870.
23. Kaczmarek, L. (2002) in *Handbook of Chemical Neuroanatomy: Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction.*, eds. Kaczmarek, L. & Robertson, H. A. (Elsevier, Amsterdam), Vol. 19, pp. 189–216.
24. McKernan, M. G. & Shinnick-Gallagher, P. (1997) *Nature* **390,** 607–611.
25. Quirk, G. J., Repa, C. & LeDoux, J. E. (1995) *Neuron* **15,** 1029–1039.
26. Rogan, M. T., Staubli, U. V. & LeDoux, J. E. (1997) *Nature* **390,** 604–607.
27. Kiyokawa, Y., Kikusui, T., Takeuchi, Y. & Mori, Y. (2005) *Brain Res.* **1043,** 145–154.
28. Bialy, M., Rydz, M. & Kaczmarek, L. (2000) *Behav. Neurosci.* **114,** 983–990.
29. Brudzynski, S. M., Ociepa, D. & Bihari, F. (1991) *J. Psychiatry Neurosci.* **16,** 221–226.
30. Beckett, S. R., Duxon, M. S., Aspley, S. & Marsden, C. A. (1997) *Brain Res. Bull.* **42,** 421–426.
31. Paxinos, G. & Watson, C. (1982) *The Rat Brain in Stereotaxic Coordinates* (Academic, New York).